22nd EMDS ANNUAL MEETING

"Diversity and Plasticity of the Innate Immune Response"

Brescia (Italy) – School of Medicine, University of Brescia

September 18th – 20th, 2008

Scientific Committee

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LIST OF INVITED SPEAKERS and CHAIRMEN

- ✓ Petronela Ancuta (Montreal, Canada)*
- ✓ Raffaele Badolato (Brescia, Italy)
- ✓ Christian Bogdan (Erlangen, Germany)
- ✓ Marco Cassatella (Verona, Italy)
- ✓ Antonio Celada (Barcelona, Spain)
- ✓ Mario Colombo (Milano, Italy)
- ✓ Marco Colonna (St. Louis, USA)
- ✓ Suzanne Crowe (Melbourne, Australia)*
- ✓ Michele De Palma (Milano, Italy)
- ✓ Charles A. Dinarello (Denver, USA)
- ✓ Frédéric Geissmann (Paris, France)*
- ✓ Sergij Goerdt (Mannheim, Germany)
- ✓ Siamon Gordon (Oxford, United Kingdom)
- ✓ Veronika Grau (Marburg, Germany)*
- ✓ Derek Hart (Brisbane, Australia)*
- ✓ Peter M. Henson (Denver, USA)
- ✓ Emilio Hirsch (Torino, Italy)
- ✓ Steffen Jung (Rehovot, Israel)*
- ✓ Michael Karin (La Jolla, USA)
- ✓ Pieter Leenen (Rotterdam, The Netherlands)*
- ✓ Yong-Jun Liu (Houston, United States)*
- ✓ Manfred Lutz (Würzburg, Germany)
- ✓ Gordon MacPherson (Oxford, United Kingdom)*
- ✓ Angelo Andrea Manfredi (Milano, Italy)

- ✓ Marcus Manz (Bellinzona, Switzerland)*
- ✓ Angelo Messina (Catania, Italy)
- ✓ Guido Poli (Milano, Italy)
- ✓ Federica Sallusto (Bellinzona, Switzerland)
- ✓ Jürgen Scherberich (München, Germany)*
- ✓ Jürgen Schmitz (Bergisch-Gladbach, Germany)*
- ✓ Ken Shortman (Melbourne, Australia)*
- ✓ Antonio Sica (Milano, Italy)
- ✓ Alexander Steinkasserer (Erlangen, Germany)
- ✓ Herbert Strobl (Wien, Austria)*
- ✓ Sofie Struyf (Leuven, Belgium)
- ✓ Mariagrazia Uguccioni (Bellinzona, Switzerland)
- ✓ Marek Zembala (Krakow, Poland)*
- ✓ Loems Ziegler-Heitbrock (Leichester, United Kingdom)

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SCIENTIFIC PROGRAMME

THURSDAY, SEPTEMBER 18th 2008

10.00 a.m.	Registration
11.30 a.m. – 12.30 p.m.	EMDS Council Meeting
01.00 p.m.	Opening Ceremony

01.15 p.m. – 03.30 p.m. 1st Scientific Session

Regulation of activation – *Chairmen: C. Bogdan - A. Messina*

01.15 p.m.	**Pro and anti-inflammatory members of the IL-1 Family** *C. Dinarello*
01.40 p.m.	**Blood monocytes in organ transplants** *V. Grau*
	Oral Presentations
02.05 p.m.	**Mincle, the macrophage inducible C-type lectin, plays a fundamental role in the innate immune response against Candida albicans** *Manzanero S., Salvage-Jones J.A., Hitchens K., Butcher S., Beckhouse A.G., Ashman R.B., Wells C.A.*
02.20 p.m.	**Increased TNF expression in CD43^{++} murine blood monocytes** *Frankenberger M., Burke B., Ahmad R., Kadioglu A., Hume D.A., Ziegler-Heitbrock L.*
02.35 p.m.	**Divergent effects of hypoxia on dendritic cell functions** *Mancino A., Schioppa T., Larghi P., Pasqualini F., Sozzani S., Mantovani A., Sica A.*
02.50 p.m.	**IL-10 and NF-kB p50 limit differentiation of monocytes to pathogenic TNF/INOS producing dendritic cells during parasitic infection** *Bosschaerts T., Guilliams M., Vandendriessche T., Sica A., Engle D., De Baetselier P., Beschin A.*

03.05 p.m. **CD300 Regulation of DC Functions**
D. Hart

03.30 p.m. – 04.00 p.m. *Coffee Break with Poster Exhibition*

04.00 p.m. – 06.25 p.m. 2nd Scientific Session

Pathways of activation *– Chairmen: R. Badolato – M. Cassatella*

04.00 p.m. **Response of phagocytes to apoptotic cells**
P.M. Henson

04.25 p.m. **Mechanisms involved in macrophage survival**
A. Celada

Oral Presentations

04.50 p.m. **mDCAR1 - Characterization of a novel member of the C-type lectin family**
Kaden S.A., Vasters K., Geilich M., Fuchs S., Schmitz J., Winkels G.

05.05 p.m. **Functional analysis of the IREM-2 receptor (CD300e) in human monocytes**
Brckalo T., Cassatella M., Lopez-Botet M.

05.20 p.m. **Modulation of the inflammatory response to non self and self ligands by the long pentraxin PTX3**
Cotena A., Maina V., Sironi M., Anselmo A., Mantovani A., Garlanda C.

05.35 p.m. **DAP12 signaling in myeloid cells**
M. Colonna

06.00 p.m. **Diversity and functions of alternatively activated macrophages**
S. Goerdt

06.45 p.m. *Buses departure from the Congress Venue to the Cloister of San Cristo (Brescia)*
Welcome Cocktail and Ceremony for the Centenary of the Nobel Prize to
Elie Metchnikoff for the discovery of Phagocytosis
Chairman: *A. Mantovani*
Keynote Lecture: **Mononuclear phagocytes: past, present and future**
S. Gordon

FRIDAY, SEPTEMBER 19th 2008

08.30 a.m. – 10.30 a.m. 3rd Scientific Session

DC in the activation of adaptive immunity – *Chairmen:A. Manfredi – L. Ziegler-Heitbrock*

08.30 a.m.	**Dendritic cell and lymphocyte migration in immunity and autoimmunity** *F. Sallusto*
08.55 a.m.	**Monocyte fates in the intestinal lamina propria** *S. Jung*
	Oral Presentations
09.20 a.m.	**The effects of alpha-1-antitrypsin on T regulatory cells: role in islet transplantation** *Mizrahi M., Toledano M., Defelice N., Dinarello C., Lewis E.*
09.35 a.m.	**Activin A induces largerhans cell differentiation** *Musso T., Castagnoli C., Vermi W., Daniele R., Salogni L., Girolomoni G., Sozzani S.*
09.50 a.m.	**Identification of a novel dendritic cell type from human lymphoid tissue expressing AIRE (autoimmune regulator)** *Poliani P.L., Marrella V., Ravanini M., Notarangelo L.D., Badolato R., Peterson P., Villa A., Facchetti F.*
10.05 a.m.	**The molecular basis of dendritic cell subsets and plasticity** *Y-J. Liu*
10.30 a.m. – 11.00 a.m.	*Coffee Break with Poster Exhibition*

11.00 a.m. – 12.20 p.m. 4th Scientific Session

Host pathogen interaction – *Chairmen: G. Poli - J. Scherberich*

11.00 a.m. **Functional heterogeneity of monocyte-derived dendritic cell subsets: relevance for CD4+ T cell differentiation and HIV pathogenesis**
P. Ancuta

11.25 a.m. **Role of monocytes in HIV pathogenesis**
S. Crowe

Oral Presentations

11.50 a.m. **The role of nitric oxide and arginase for the NO-synthase-mediated killing of intracellular leishmania parasites in macrophages**
Schleicher U., Koenig T., El-Gayar S., Valdez C.A., Keefer L.K., Murray P., Bogdan C.

12.05 p.m. **HIV-1 matrix protein p17 induces human plasmacytoid dendritic cells to acquire a migratory immature cell phenotype**
Fiorentini S., Riboldi E., Facchetti F., Giagulli C., Fabbri M., Sozzani S., Caruso A.

12.20 p.m. – 01.30 p.m. *Buffet Lunch with Poster Exhibition*

01.30 p.m. – 04.00 p.m. 5th Scientific Session

Heterogeneity – *Chairmen: M. De Palma – A. Steinkasserer*

01.30 p.m. **Functional dichotomy of plasmacytoid dendritic cells: antigen specific activation of T cells versus production of type I interferon**
J. Schmitz

01.55 p.m. **Dendritic cell development from hematopoietic progenitor cells**
M. Manz

02.20 p.m. **The development of different types of spleen dendritic cells**
K. Shortman

02.45 p.m. **Development and functions of monocytes**
F. Geissmann

03.10 p.m.	**Molecular mechanisms in human dendritic cell subset differentiation** *H. Strobl*
03.35 p.m.	**Involvement of microRNAs in the regulation of DC maturation** *P. Leenen*
04.00 p.m.	*Coffee Break*

04.00 p.m. – 06.00 p.m. Poster Session

in parallel session (reserved to DC-THERA Members – session restricted by invitation)

NOMENCLATURE WORKSHOP DC-THERA/EMDS

06.00 p.m.	***Lecture BD Prize 2008*: Monocytes and Macrophages: central mediators of antibody mediated pro- and anti-inflammatory responses** *F. Nimmerjahn*
06.30 p.m.	EMDS General Assembly
07.30 p.m.	*Buses departure from the Congress Venue to "Relais Franciacorta" (Colombaro di Cortefranca, Brescia)*
08.00 p.m.	*Social Dinner at "Relais Franciacorta"*

SATURDAY, SEPTEMBER 20th 2008

08.30 a.m. – 10.45 a.m. 6th Scientific Session

Migration – *Chairmen: M. Lutz – M. Uguccioni*

08.30 a.m. **Regulation of chemokine activity by proteases**
S. Struyf

08.55 a.m. **Migrating intestinal dendritic cells and intestinal immune responses**
G. MacPherson

Oral Presentations

09.20 a.m. **Proliferation and migration of macrophages is enhanced by sphingosine-1-phosphate (S1P) and pFTY720**
Lucke S., Keul P., Von Wnuck Lipinski K., Levkau B.

09.35 a.m. **Defective T cell education by dendritic cells contributes to impaired antigen-specific immunity in Wiskott-Aldrich syndrome**
Bouma G., Mendoza-Naranjo A., Burns S., Thrasher A.

09.50 a.m. **P-glycoprotein is essential for dendritic cells differentiation, development and T cells polarization**
Lee J.S., Jung I.D., Jeong Y.I., Chang J.H., Lee C.M., Kim M.J., Park Y.M.

10.05 a.m. **Lactoferrin, a major defense protein of innate immunity, is a novel maturation factor for human dendritic cells**
Spadaro M., Caorsi C., Ceruti P., Varadhachary A., Forni G., Pericle F., Giovarelli M.

10.20 a.m. **Effects of PI3K signaling driving leukocyte migration**
E. Hirsch

10.45 a.m. – 11.15 a.m. *Coffee Break with Poster Exhibition*

11.15 a.m. – 01.05 p.m. 7th Scientific Session

Cancer-related inflammation – *Chairmen: M. Colombo - M. Zembala*

Oral Presentations

11.15 a.m. **Molecular characterization of Tie2-expressing monocytes (TEMs): relationship with tumor-associated macrophages**
Pucci F., Venneri M.A., Di Serio C., Naldini L., De Palma M.

11.30 a.m. **Apoptotic cells stimulate PPARgamma thereby transrepressing NFkappaqB**
Jennewein C., Kuhn A.M., Schmidt M.V.,Meilladec-Jullig V.,Von Knethen A., Brüne B.

11.45 a.m. **Identification of discrete tumor-induced myeloid-derived suppressor cell subpopulations with distinct T-cell suppressive activity**
Van Ginderachter J., Van den Bossche J., Van den Bergh R., Beschin A., De Baetselier P.

12.00 p.m. **Hypoxia Transcriptionally Induces CCL-20 in Primary Human Monocytes Through NF-kBp50**
Varesio L., Bosco M.C., Battaglia F., Delfino S., Gattorno M., Puppo M.

12.15 p.m. **Role of inflammation and inflammatory cells in cancer metastasis**
M. Karin

12.40 p.m. **Polarized inflammation and tumor progression**
A. Sica

01.05 p.m. Closing Remarks

01.30 p.m. – 02.30 p.m. Buffet Lunch

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Bosschaerts Tom	*Thursday, Sept. 18th*	IL-10 and NF-kB P50 limit differentiation of monocytes to pathogenic TNF/INOS producing dendritic cells during parasitic infection
Bouma Gerben	*Saturday, Sept. 20th*	Defective T cell education by dendritic cells contributes to impaired antigen-specific immunity in Wiskott-Aldrich syndrome
Brckalo Tamara	*Thursday, Sept. 18th*	Functional analysis of the IREM-2 receptor (CD300e) in human monocytes
Celada Antonio	*Thursday, Sept. 18th*	Mechanisms involved in macrophage survival
Colonna Marco	*Thursday, Sept. 18th*	DAP12 signaling in myeloid cells
Cotena Alessia	*Thursday, Sept. 18th*	Modulation of the inflammatory response to non self and self ligands by the long pentraxin PTX3
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Dinarello Charles A.	*Thursday, Sept. 18th*	Pro and anti-inflammatory members of the IL-1 Family
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Giovarelli Mirella	*Saturday, Sept. 20th*	Lactoferrin, a major defense protein of innate immunity, is a novel maturation factor for human dendritic cells
Goerdt Sergij	*Thursday, Sept. 18th*	Diversity and functions of alternatively activated macrophages
Gordon Siamon	*Thursday, Sept. 18th*	Keynote Lecture - Mononuclear phagocytes: past, present and future
Grau Veronika	*Thursday, Sept. 18th*	Blood monocytes in organ transplants

Hart Derek	*Thursday, Sept. 18th*	CD300 Regulation of DC Functions
Henson Peter M.	*Thursday, Sept. 18th*	Response of phagocytes to apoptotic cells
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Karin Michael	*Saturday, Sept. 20th*	Role of inflammation and inflammatory cells in cancer metastasis
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Varesio Luigi	*Saturday, Sept. 20th*	Hypoxia Transcriptionally Induces CCL-20 in Primary Human Monocytes Through NF-kBp50

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P.019	*Coccia Eliana Marina*	Plasmacytoid dendritic cells in multiple sclerosis: intracerebral recruitment and impaired maturation in response to interferon-ß.
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P.105	*Schleicher Ulrike*	The role of nitric oxide and arginase for the no-synthase-mediated killing of intracellular leishmania parasites in macrophages
P.106	*Siedlar Maciej*	Intravenous immunoglobulins diminish the number of proinflammatory CD14+CD16+ monocytes in peripheral blood of CVID patients.
P.107	*Lucke Susann*	Proliferation and migration of macrophages is enhanced by sphingosine-1-phosphate (S1P) and pFTY720
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P.112	*Mucci Ilaria*	The methodological approach for the generation of human dendritic cells from monocytes affects the maturation state of resultant dendritic cells
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P.122	*Engel Daniel*	CCR2 mediates homeostatic and inflammatory release of Gr1HI monocytes from the bone-marrow, but is dispensable for bladder infiltration in bacterial urinary tract infection
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P.125	*Ribechini Eliana*	The Gr-1 (RB6-8C5) antibody is not depleting but induces differentiation of macrophages and thereby ablates myeloid-derived suppressor cell activity
P.126	*Pucci Ferdinando*	Molecular characterization of Tie2-expressing monocytes (TEMs): relationship with tumor-associated macrophages
P.127	*Sredni Benjamin*	The novel non-toxic tellurium compound SAS blocks angiogenesis by inhibition of cell migration and adhesion: interference with αvβ3 activity.

ABSTRACTS OF INVITED PRESENTATIONS

(in order of presentation)

Pro- and anti-inflammatory members of the IL-1 family

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The IL-1 family of cytokines comprises 11 members: IL-1a (IL-1F1), IL-1b (F2), IL-1Ra (F3), IL-18 (F4), IL-1F5 to 10 (F5 to 10), and IL-33 (F11). Whereas the receptors, signaling pathways, and functions of the classical members of the family have been studied extensively, knowledge of the more recently described IL-1 family members F5 to 10, which were identified by gene searches, remains limited. IL-33 (IL-1F11) stands apart from this latter group, as this family member is the long-sought ligand for the IL-1 receptor family member ST2. IL-1F7 also shares the structural pattern of the IL-1 family and IL-1F7 mRNA is present in various tissues and in PBMC and DC. The protein was detected in human monocytes. Of the 5 splice variants, IL-1F7b is the only variant which contains 5 of the 6 exons. IL-1F7b have been shown to bind to the IL-18 receptor a chain (IL-18Ra)but a wide range of concentrations of recombinant IL-1F7b does not exert any IL-18-agonistic properties. Overexpression of F7b in mouse RAW macrophages reduced LPS-induced cytokines and that mature IL-1F7b translocates from the cytoplasm to the nucleus via caspase-1-dependent processing. There is a role for endogenous IL-1F7. In freshly obtained PBMC, reducing F7b protein levels with siRNA resulted in increased production of over 15 LPS-induced pro-inflammatory cytokines. We conclude that F7b functions to reduce inflammation induced by several TLR ligands and by IL-1b itself.

BLOOD LEUKOCYTES IN ORGAN TRANSPLANTS

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Host leukocytes encounter allogeneic vascular endothelial cells as early as during reperfusion of an organ transplant. Their interactions are decisive for the fate of the graft: 1) Endothelial cells constitutively express MHC class I and upon stimulation MHC class II antigens (MHCII) and directly present antigen to host T cells. 2) Attack of graft endothelial cells by blood leukocytes, may lead to acute graft rejection or to continuous damage eventually resulting in chronic rejection which is mainly a vascular disease. 3) Graft infiltration is at least partially controlled by interaction of blood leukocytes with endothelial cells. 4) Organ-specific differences in the rejection of vascularised grafts are possibly mediated by functional heterogeneity of blood vessel endothelium.

We analysed intravascular graft leukocytes in a fatal rat model of acute renal allograft (Dark Agouti to Lewis) rejection. About 90 million leukocytes were present in blood vessels of allografts, whereas only about 10 million cells were present in isograft and 5 million cells in normal kidneys. Allograft cells expressed increased amounts of tissue factor, inducible NO synthase and TNF-alpha, factors which are probably involved in graft destruction. About 14% of the intravascular allograft leukocytes were T cells and 73% were monocytes which exerted cytotoxicity in vitro. Most intravascular allograft monocytes were MHCIIpos, CD4neg, CD11alow, CD18low, CD43low, CD62Lpos, CD71pos, and CD161int. This pattern of cell surface antigens is characteristic for activated rat monocytes. Most isograft monocytes exhibited a resting phenotype MHCIIneg, CD62Lneg, CD71neg, CD161neg, CD4pos, CD11ahigh, CD18high, and CD43high.

After pulmonary transplantation (Dark Agouti to Lewis), only about 34 million leukocytes accumulated in allograft vessels. In contrast to renal allografts, 50% of these cells were monocytes which expressed an unusual pattern of cell surface antigens: The levels MHCII and CD161 were increased but the expression of all other markers resembled resting monocytes. Although pulmonary allografts are readily destroyed, the cytokine mRNA expression pattern of graft leukocytes rather suggested tolerogenic functions: IFN-gamma, IL-1beta and IL-10 mRNA expression was increased in allograft leukocytes compared to isograft cells, IL-12 mRNA was decreased and IL-2, IL-6, TNF-alpha, as well as TGF-beta mRNA did not change.

In conclusion, during acute rejection of organs transplants, dramatic, organ-specific changes occur in the intravascular leukocyte population. Most of these cells are monocytes which seem to destroy renal allografts but may induce tolerance in lung allograft recipients. The elucidation of the function of intravascular leukocytes will improve our understanding of the pathogenesis of acute and chronic allograft rejection and may result in novel therapeutic options which are much-needed.

The CD300 Molecules Regulate Dendritic Cell and Monocyte Functions

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The regulation of dendritic cell (DC) response is essential to maintain the host's wellbeing, for example, the plasmacytoid (pDC) IFNα response needs to be self-limited to prevent the inappropriate responses seen in chronic inflammatory and autoimmune diseases. Failure to regulate pDC IFNα production may contribute to aberrant immune responses and diseases such as psoriasis. The CD300 family of leukocyte surface molecules includes six immunoregulatory glycoproteins that have the ability to positively and negatively balance an immune response. The CD300a and CD300c molecules are expressed on pDC and CD300a is one of the few molecules with cytoplasmic immunotyrosine based inhibitory motifs known to be expressed on pDC. We therefore investigated the contribution of the CD300a and CD300c molecules to the regulation of pDC IFNα production. On normal pDC, CD300a and CD300c molecules were down-regulated by both TLR7 and TLR9 ligands. We showed that exogenous IFNα also down-regulated CD300a/c expression in pDC. This suggested that the TLR ligand induced down-regulation of CD300a/c might be an indirect effect. In subsequent experiments, we added neutralizing antibody to IFNα and found that it abolished the CpG-ODN induced CD300a/c down-regulation. The effect of CD300a and CD300c activation on the function of pDC was investigated by cross-linking them on pDC with the CD300a/c monoclonal antibody, CMRF-35. This had no effect on CD80, CD83 and CD86 expression, but decreased MHC-II expression. Significant reductions in pDC TNFα and IL-6 production occurred after CMRF-35 cross-linking, however, in marked contrast, CpG-ODN induced IFNα was increased in pDC cross-linked with CMRF-35. In contrast, IFNα production by pDC from patients with the chronic inflammatory disease psoriasis was decreased by CMRF-35 crosslinking during CpG activation. The immune regulators CD300a and CD300c regulate pDC cytokine production and in turn, the pDC released cytokines regulate CD300a/c expression. This feedback regulation pathway in pDC involving CD300a/c is abnormal in psoriasis and may contribute to the disease pathogenesis. Likewise CD300e is expressed on monocytes and was shown to influence their function. Our recent data suggests that CD300f plays an active role in modulating cell migration and new data on its regulatory role in immune responses will be presented.

RESPONSE OF PHAGOCYTES TO APOPTOTIC CELLS

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Phagocytic removal of cells undergoing various forms of programmed cell death is an essential and highly conserved metazoan process that can be mediated not only by professional phagocytes such as macrophages and dendritic cells but also by numerous other cell types. In the case of apoptosis, a number of surface ligands are expressed and/or rearranged on the cell surface that are in turn engaged by a plethora of bridge molecules and potential receptors on the phagocyte surface. At least two unique, but intersecting signal pathways are involved in the uptake, which is critically regulated by a balance between different Rho family GTPases. An important element of the cell removal is that it is generally silent, i.e. not accompanied by local inflammatory or immunological responses. Thus, a second set of responses to apoptotic cells involves suppression of inflammation and immunity. A third category of response includes the generation of growth maintenance factors, which, we hypothesize, may contribute to normal tissue homeostasis by contributing to local cell survival or replenishment. Important to each of these sets of effects is recognition of phosphatidylserine (PS) exposed on the outer leaflet of the apoptotic cell. However, PS also becomes exposed during many forms of cell activation, even in the absence of programmed cell death, which suggests additional levels of regulation that can distinguish between viable and apoptotic cells – sometimes called "don't eat me signals".

MECHANISMS INVOLVED IN MACROPHAGE SURVIVAL

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Macrophages, like other cells of the immune system, are produced in large amounts and most die through apoptosis. These phagocytic cells survive in the or extracellular matrix proteins,γpresence of soluble factors, such as IFN- like decorin. The mechanism toward survival requires the blocking of proliferation at the G1/S boundary of the cell cycle, which is mediated mediated by the cyclin-dependent kinase (cdk) inhibitor, p27kip and the induction of a , LPS),γcdk inhibitor, p21waf1. During the process of classical activation (IFN- macrophages produce toxic molecules that lead to damage. At the same time, these cells show high transcriptional activity, during which more than 400 genes are induced. We have found that ruptures of single or double strand DNA occur during classical activation and have identified several of the mechanisms induced triggered to repair the DNA damage caused during this type of activation.

DAP12 SIGNALING IN MYELOID CELLS

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Diversity and functions of alternatively activated (M2) macrophages

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The concept of alternative activation of macrophages was developed corresponding to the Th1/Th2 paradigm by S. Gordon's and our groups and focused primarily on IL-4-induced effector functions of alternatively activated macrophages. By a tremendously increasing cohort of experimental studies, the concept has gained momentum and has been significantly diversified. It has been shown for example that alternatively activated macrophages regulate infectious (esp. parasite) and tumor immunity, control transplant rejection and metabolic diseases (such as diabetes, obesity and atherosclerosis) and are indispensable in the various phases of resolving inflammation and wound healing. Besides IL-4, mediators such as IL-10, TGF-β and glucocorticoids have been shown to induce different activation states of M2 macrophages leading A. Mantovani to propose a concept of M2a, M2b, and M2c alternatively activated macrophages. Our group has attributed functional processes such as matrix remodelling, tissue clearance, and anti-inflammatory signalling to different M2 macrophage subsets and has identified several novel molecules that exert these specialized effects including CCL-18, stabilin-1 and IL-17B receptor. Beyond Th1/Th2 cells, the interactions of novel T-cell subsets such as Th17 cells or Tregs with macrophages in the induction of novel alternative macrophage differentiation pathways and effector functions have not been thoroughly explored. In addition, malignant epithelial tumours that are often macrophage-rich seem to induce either an M2 or a mixed M2/M1 macrophage phenotype. Data about the effects of non-malignant pro- or anti-inflammatory epithelial cells on macrophage differentiation are also lacking, but are crucial to understand immune regulation in healthy and inflamed skin and mucosal immune systems.

THE MACROPHAGE: PAST, PRESENT AND FUTURE

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Two thousand and eight marks the Centenary of Elie Metchnikoff's Nobel Prize, jointly with Paul Ehrlich. The discovery of phagocytosis as a host leukocyte defence mechanism is attributed to his research on simple organisms in Messina, following the tradition of marine biology at Naples. His subsequent career at the Pasteur Institute confirmed his reputation as an innovative experimental pathologist.

The modern era of macrophage cell biology started with the in vitro studies by Zanvil Cohn and his colleagues at Rockefeller University in the 60's, followed by the rapid characterisation of macrophage receptors, products and antimicrobial functions. I shall review selected aspects dealing with cell heterogeneity, recognition and activation.

Future goals are to dissect the dual nature of macrophage functions in homeostasis and innate immunity, to uncover further mechanisms of dysregulation, and to target macrophages and their products selectively in humans.

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DENDRITIC CELL AND LYMPHOCYTE MIGRATION IN IMMUNITY AND AUTOIMMUNITY

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DENDRITIC CELLS FUNCTIONS REVEALED BY THEIR CONDITIONAL AND CONSTITUTIVE IN VIVO ABLATION

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The functional units of multi-cellular organisms consist arguably of cells, rather than single transcription units. This provides the rationale for cell ablation as opposed to gene ablation as experimental approach to study complex physiological phenomena. When applied to dendritic cells (DC) cell ablation aims to functionally define DC and DC subsets, which are still largely characterized by differential surface marker expression and anatomic location, in context, i.e. the intact organism.

Here we will report on recent findings of unexpected physiological DC functions beyond their established role as unique antigen presenting cells. We will present the characterization of perivascular clusters of BM-resident dendritic cells (bmDC) that wrap blood vessels and are seeded with lymphocytes. We propose that bmDC define novel "BM immune niches" and are critical players in the initiation of T and B cell responses towards blood-borne antigens in the BM. Moreover, conditional ablation revealed the surprising requirement of bmDC for the survival of re-circulating mature B cells [1]. Finally we will report the characterization of a novel binary transgenic mouse strain [2, 3] that lacks CD11chigh conventional DC (cDC) due to constitutive cell-type specific expression of a suicide gene.

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THE MOLECULAR BASIS OF DENDRITIC CELL SUBSETS AND PLASTICITY

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FUNCTIONAL HETEROGENEITY OF MONOCYTE-DERIVED DENDRITIC CELL SUBSETS: RELEVANCE FOR CD4+ T CELL DIFFERENTIATION AND HIV PATHOGENESIS

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Human peripheral blood monocytes include subsets with distinct phenotype, function, and trafficking that are likely to give rise to functionally distinct RIII+ monocytesγmonocyte-derived dendritic cell (MDDC) subsets. CD16/Fc represent 5-10% of circulating monocytes in healthy individuals and are dramatically expanded in several pathological conditions including sepsis, cancer, and human immunodeficiency virus type 1 (HIV-1) infection. CD16+ monocytes produce high levels of pro-inflammatory cytokines (i.e., IL-1, TNF, and IL-6) and promote HIV-1 replication in resting CD4+ T cells upon) by producing the CCR3 and CCR4 ligandsΦdifferentiation into macrophages (M CCL24, CCL2, CCL17, and CCL22. CD16+ monocytes express CX3CR1 and have the capacity to migrate into tissues expressing the CX3CR1 ligand CX3CL1 under constitutive and inflammatory conditions, including the brain and gut-associated lymphoid tissues, which are major sites for HIV replication in vivo. To explore the differentiation potential and function of monocyte subsets with relevance for HIV-1 pathogenesis, we performed a genome wide analysis of differential gene expression in CD16+ and CD16- monocytes isolated from peripheral blood of healthy individuals. The upregulation of CD43, sialic acid binding Ig-like lectin 10 (SIGLEC10), LFA-1, CXCL16, C3AR1, colony stimulating factor 3 receptor (CSF3R), tryptophanyl-tRNA synthetase (WARS), retinoic acid receptor, alpha (RARA), and v-maf musculoaponeurotic fibrosarcoma oncogene homolog B (MafB), RI, CCR1, CCR2, formyl peptideγtogether with the downregulation of CD14, CD64/Fc receptor 1 (FPR1), C1QR1, CSF1R, and myeloid cell nuclear differentiation antigen (MNDA) demonstrated that CD16+ monocytes are at a more advanced stage of - and DC-like transcription program. BothΦmonocyte differentiation with a more M CD16+ and CD16- monocytes differentiated into immature DC (i.e., CD14-CD1a+CD1c+DC-SIGN+) when cultured in the presence of GM-CSF and IL-4, acquired a mature DC phenotype upon LPS stimulation (i.e., CD83+CCR7+), and induced similar autologous CD4+ T cell proliferation when loaded with SEB superantigen. However, CD16+ compared to CD16- MDDC produced higher levels of CCR4 ligands and promoted higher levels of HIV-1 replication in CD4+ T cells. Two hypotheses are currently under investigation to explain preferential HIV replication in CD16+ MDDC:CD4+ T cell cocultures: (1) CD16+ MDDC have a greater ability to capture and transmit HIV to CD4+ T cells and (2) CD16+ MDDC trigger a differentiation program in CD4+ T cells that increases susceptibility to HIV infection. CCR4, CCR6, and CXCR3 are being used as markers for Th1 (T-bet+/IFN-g+), Th2 (GATA-3+/IL-4+), and Th17 (RORC+/IL-17+) lineage differentiation. Finally, I will discuss the potential link between an increased frequency of circulating CD16+ monocytes in HIV-infected patients and specific depletion of CCR6+CD4+ T cell subsets, which are highly permissive for HIV infection in vivo.

ROLE OF MONOCYTES IN HIV PATHOGENESIS

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We have shown that HIV-1 can be detected in monocytes isolated from HIV infected patients with prolonged viral suppression and that these cells are productively infected.. As monocytes are generally refractive to HIV infection in vitro we hypothesised a subset of monocytes harbours the majority of HIV in vivo. The CD14lo/CD16hi subset is reportedly expanded in HIV-infected individuals, however their relevance to infection has not been established. The minor CD14variable/CD16+ monocyte subset and the major CD14hi/CD16- subset were purified from PBMCs from HIV infected patients. We found that in the majority of patients HIV DNA could only be detected in the CD14variable/CD16+ monocyte population. These data were also confirmed with in vitro experiments. Using a highly sensitive nested PCR for HIV-specific gag DNA as well as real time PCR, we compared the amount of HIV DNA found within the monocyte subsets in blood to that found in CD4+ memory T cells, a well studied reservoir of HIV. The difference between DNA levels in the CD14variable/CD16+ monocytes and the memory T cells was not statistically significant and levels were significantly greater than in Cd14+/CD16- monocytes.. Analysis of the monocyte subsets for expression of CD4, CCR5 and CXCR4 showed CD14variable/CD16+ monocytes express more CD4 and CCR5 which may account for the decreased susceptibility of the CD14 subset to HIV entry. However as an additional mechanism the CD14hi/CD16- subset expressed the intracellular post-entry restriction factor APOBEC3G exclusively in the low molecular weight active form whereas the CD 14 variable/CD16+ monocyte subset expressed APOBEC3G in predominantly a high/intermediate molecular weight inactive forms. Although many questions are still unanswered, there is increasing recognition that latent T cell reservoirs cannot fully explain the failure of HAART to eradicate HIV-1 and that monocyte/macrophages play a critical role as a source of residual infection in patients treated with highly active antiretroviral therapy. These CD16+ monocytes have high migratory ability and may play a major role in the establishment of HIV infection in the brain and the development of HIV associated dementia.

FUNCTIONAL DICHOTOMY OF PLASMACYTOID DENDRITIC CELLS: ANTIGEN PRESENTATION VS. PRODUCTION OF TYPE I INTERFERON

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Plasmacytoid dendritic cells (PDC) also referred to as type I interferon producing cells (IPC) are believed to act as a link between innate and adaptive immunity by producing type I interferon, and subsequently triggering adaptive T cell mediated immunity by differentiating into mature DC. However, it remains controversial to which degree PDC play a direct role as antigen presenting cells (APC) in the activation of naïve and memory CD4+ and CD8+ T cells and to which degree APC functions and production of type I interferon are directly linked.

To analyze whether human and mouse PDC can act as APC, we decided to adopt a strategy involving antibody mediated targeting of antigen to PDC-specific receptors: BDCA-2 (CD303) for human PDC and mPDCA-1 (BST-2, CD317) for murine PDC. Independent on Toll-like receptor (TLR) ligand stimulation antigen is rapidly endocytosed by these receptors and traffics via early sorting endosomes to emerging MHC-enriched compartments (MIIC). In vitro restimulation of human CMV-specific CD4+ effector memory T cells and in vitro priming of murine naïve ovalbumin (OVA)-specific T-cell receptor transgenic CD4+ and CD8+ T cells, however, are dependent on appropriate TLR ligand stimulation of PDC.

Most interestingly, at least in human PDC processing and presentation of CMV antigen and production of type I interferon are mutually exclusively induced by distinct CpG oligonucleotides. Type B CpG oligonucleotide (CpG-B)-stimulated PDC efficiently process and present CMV antigen and are thus capable of stimulating CMV-specific CD4+ effector memory T cells. CpG-A stimulated PDC produce large amounts of type I interferon and express programmed death-1 ligand 1 (PD-1L), a molecule which is known to inhibit T cell activation via PD-1 ligation. CpG-A plus CpG-B co-stimulated PDC behave like CpG-B stimulated PDC, indicating that CpG-B induction of antigen processing and presentation in PDC concurrently inhibits type I interferon production.

Our results suggest that innate and adaptive immunity are not linked at the level of individual PDC which first produce type I interferon and then differentiate in mature DC, but rather at the population level, where depending on the stimulation received, individual PDC either contribute to the innate response by production of IFN-alpha or to the adaptive response by antigen presentation and stimulation of T cells.

REGULATION OF DENDRITIC CELL HOMEOSTASIS

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Dendritic cells (DCs) are a heterogeneous group of professional antigen presenting cells that belong to the hematopoietic system and are located in lymphoid and non-lymphoid tissues throughout the body. Most lymphoid-tissue steady-state DCs are end-stage divided cells with half-lives of only few days. As other hematopoietic cells, DCs derive from self-renewing hematopoietic stem cells (HSCs) that via a unidirectional, hierarchical differentiation process give rise to subsequent progenitors and mature cells. This differentiation process is associated with the loss of self-renewal capacity at an early multi-potent progenitor level, followed by restriction to lineage committed progenitors with still maintained, but gradually reduced proliferation potential. In order to study the regulation of this differentiation process, we recently isolated highly cycling, common dendritic cell progenitors (CDPs) from mouse bone marrow that are restricted to the DC lineage, and efficiently produce plasmacytoid (pDC), as well as conventional DC (cDC) offspring in vitro and in vivo. In steadystate, HSC to CDP differentiation takes about 3-4 days, while CDP to DC differentiation takes about one week, and both sequential developmental steps require about equal numbers of cellular divisions. Expression (natural and artificial) of the receptor tyrosine kinase flt3, as well as the analysis of stem and progenitor cell transferred into mice with high levels or absent Flt3L demonstrate that both steps, DC lineage commitment and thus DC progenitor generation, as well as subsequent cellular expansion and dendritic cell development depend on Flt3-signalling. Furthermore, stimulation of progenitors by cytokines as M-CSF and GM-CSF, and the ligation of TLRs expressed on DC progenitors impact on both lineage sub-specialization as well as peripheral homing of DC precursors, revealing a complex interaction of steady-state and inflammatory stimuli that in concert regulate DC homeostasis.

THE DEVELOPMENT OF DIFFERENT TYPES OF SPLEEN DENDRITIC CELLS

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The dendritic cell (DC) system includes a range of specialised DC subtypes. These can be grouped into the interferon-producing plasmacytoid cells (pDC), the classical migratory DC which collect antigen in peripheral tissues then transit to lymph nodes to present antigen to T cells, the lymphoid tissue resident DC which collect and present antigen entirely within one lymphoid organ, and inflammatory DC which are not present in steady-state and only develop in response to inflammation. Whereas the immediate precursors of inflammatory and migratory DC seem to be blood monocytes, the pDC and lymphoid tissue resident conventional DC (cDC) of spleen have a different origin.

The pDC are formed in bone marrow and migrate as such through the blood stream to the spleen. The cDC of the spleen derive from a non-monocyte pre-DC precursor, which migrates from the bone marrow and then expands and finishes development in the spleen, to form the CD8+ and CD8– cDC subtypes of the mouse. The initial commitment to DC production, and to DC subtype, occurs at an intermediate stage of haematopoiesis in bone marrow. The different pathways and precursors involved, and their relationship to the monocyte-macrophage lineages, are currently being explored in several laboratories. Some of the recent and sometimes conflicting findings will be reviewed.

HETEROGENEITY OF MOUSE AND HUMAN MONOCYTES

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The immune system cellular response to tissue damage and infection requires the recruitment of blood leukocytes in the target tissue. In addition, leucocytes also plays important roles in the homeostasis of the blood vessel wall. As our understanding of monocyte biology improves, these cells appear more and more important in the general field of inflammation and homeostasis, and the issue of monocyte heterogeneity become relevant to the pathophysiology of inflammatory diseases.

Mouse monocytes originate from a bone marrow progenitor, the MDP, that also gives rise to conventional dendritic cells, through a separate differentiation pathway. Evidence accumulate that blood monocytes consist in several functional subsets. A classical CD115+ CX3CR1low CCR2+ subset (Gr1+ in the mouse, that probably correspond to CD14+ monocytes in human), has been studied and by numerous investigators, but may still contain several functional populations. A second CD115+ CX3CR1high CCR2- subset (Gr1- in the mouse, that probably correspond to CD14dim CD16+ monocytes in human) have recently been shown to patrol blood vessels in the steady state, and to extravasate during infection with Listeria monocytogenes or in the healing myocardium. We will discuss efforts to further characterize this population of patrolling monocytes in mouse and human. As the field of monocyte biology develop rapidly, new evidence suggests that additional functional subsets may be characterized.

The numbers of these subsets, the correspondance between human and mouse subsets, and whether separate subsets originate independently in the bone marrow from a common progenitor, or represent discrete stages of maturation remain, in our view, open questions.

MOLECULAR MECHANISMS IN HUMAN DENDRITIC CELL SUBSET DIFFERENTIATION

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Human DCs comprise at least three different subsets that arise via distinct immediate cell stages from hematopoietic progenitor cells. Two of these DC subsets, i.e. Langerhans cells (LCs) and monocyte-derived interstitial/dermal DCs (moDCs) arise from shared monocytic precursors. Tissue microenvironmental signals have been recognized as critical factors in regulating DC subset differentiation. However, the transcriptional mechanisms underlying these processes remained poorly defined. We here used well-defined cytokine-dependent in vitro differentiation culture models in conjunction with retroviral/lentiviral gene transduction experiments for identifying critical transcriptional regulators of human DC subset differentiation. This approach revealed the following regulatory molecules. (1) RelB. Ectopic expression of RelB forces the transition of early CD14+CD11b- to late CD14+CD11b+ monocytes. In line with this, loss of function analyses revealed that moDCs but not LCs nor plasmacytoid DCs require RelB for their development. In line with this, nuclear RelB is consitutively present in moDCs but not in LCs. (2) Factors downstream of TGF-b1 during LC subset differentiation. The cytokine TGF-b1 is strictly required for LC differentiation. When added to DC generation cultures of CD34+ progenitor cells, TGF-b1 induces LC differentiation from early monocytic cells at the expense of CD14+CD11b+ monocyte generation. Using gene chip analyses, we identified the vitamin D3 receptor (VDR) as a rapidly induced downstream target of TGF-β1 during LC lineage commitment. In line with this, ectopic VDR promoted LC generation in a TGF-b1-dependent manner. Furthermore, skin-physiologic (low) levels of vitamin-D3 (VD3) promoted LC generation, and ligation of the VDR heterodimerization partner RXRa similarly augmented LC generation in defined serum-free medium. (3) GATA-1. Using a functional genetic cDNA library screening approach, we recently identified the transcription factor GATA-1 as a repressor of VDR in monocytic cells. Interestingly, VDR is repressed during IL-4-dependent moDC differentiation. We found that GATA-1 is induced by IL-4 in moDCs, but is not detectable in LCs. Forced inducible expression of GATA-1 mimics IL-4 in re-directing moDC differentiation, and vice versa GATA-1 knock-down arrests moDC differentiation at the VDR+ Mo stage. Moreover, GATA-1 stabilizes the moDC phenotype by preventing reprogramming of DCs to monocytic cells in the presence of VD3. Therefore, GATA-1-mediated repression of VDR enables IL-4-dependent moDC subset differentiation from monocytes. Therefore, RelB, VDR and GATA-1 represent key regulators of DC subset differentiation from monocytic cells. These transcription factors might be differentially involved in the generation of DCs under steady state versus inflammatory conditions and therefore represent candidate targets for therapeutic immunomodulation.

INVOLVEMENT OF MICRORNAS IN THE REGULATION OF DENDRITIC CELL MATURATION

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The functional capacities of dendritic cells (DC) are largely determined by their state of maturation. Mature DC (mDC) can stimulate effector T cells, whereas immature DC (iDC) may induce tolerance. This functional distinction is reflected in the phenotypic characteristics of the cells and differences between iDC and mDC are determined at the level of gene expression. Recently, so-called microRNAs have been identified as important post-transcriptional regulators that determine the stability and translation of mRNAs by binding to their 3' untranslated regions. These small non-coding RNAs (approximately 22 nt) appear to be intimately involved in regulating hematopoietic cell differentiation. Therefore, we hypothesized that altered microRNA profiles might play a crucial role in the regulation of DC maturation. To approach this experimentally, we generated DC from mouse BM with GM-CSF. On day 8, different DC subsets (precursors, iDC, mDC) were isolated, based on differential CD86, CD11c and MHC class II expression. Total RNA was isolated and used for LNA-based miRNA profiling of different DC subsets.

A total of 28 miRNAs were found to be differentially expressed (minimum 1.5-fold change difference, $p<0.05$) between iDC and mDC. Of these, miRNA-300 was expressed ~5-fold higher in iDC, whereas miRNA-155 was upregulated 10-fold in mDC and even 17-fold in mDC stimulated overnight with LPS. In search of a putative target of miR-155 in DC, we found that the CSF-1 receptor (CD115) mRNA 3'UTR contains a functional binding site for miR-155. In line with this, we observed that CD115 expression strongly declines upon iDC to mDC maturation.

Taken together, our results demonstrate that miRNA profiling can serve as a cellular fingerprint in order to identify different stages of DC development, which are known to be functionally different. Thus, tolerogenic and immunogenic DC express distinct miRNAs, suggesting that differential miRNA-based regulation contributes to DC maturation at the molecular level.

MONOCYTES AND MACROPHAGES: CENTRAL PLAYERS IN ANTIBODY-MEDIATED PRO- AND ANTI-INFLAMMATORY RESPONSES

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A productive immune response results from the effective integration of positive and negative signals that have an impact on both innate and adaptive immune cells. Monocytes and Macrophages are key players in defending the host against invading pathogens. In addition, they are also involved in causing tissue damage during autoimmune disease. Activating and inhibitory Fc-receptors expressed on myeloid cells link the adaptive immune response to the innate immune system by regulating monocyte and macrophage activation. When positive signals dominate, cell activation and pro-inflammatory responses ensue, thereby providing for the elimination of pathogenic microorganisms and viruses. In the absence of such productive stimulation, cell activation is blocked or active anti-inflammatory responses can occur. Modulation of this binary system occurs through the action of cytokines, downstream signalling pathways and cell–cell contact. Perturbing these thresholds can result in aberrant responses that are either insufficient to deal with pathogenic microorganisms or result in the loss of tolerance and the induction of autoimmune responses. In this presentation we will discuss how immunoglobulin G antibodies mediate their activity via innate immune effector cells and how antibody glycosylation variants impact the outcome of the response.

REGULATION OF MONOCYTE MIGRATION THROUGH POST-TRANSLATIONAL MODIFICATION OF CHEMOKINES

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Posttranslational proteolytic processing of chemokines is a natural mechanism to regulate inflammation. Indeed, NH2-terminal truncation of SDF-1/CXCL12, by CD26 has drastic effects on its biological activities: leukocyte attraction, inhibition of HIV-infection, mobilization of stem cells. We have recently discovered a novel post-translational modification of the chemokine SDF-1/CXCL12, resulting in impaired CXCR4 binding and signaling properties. However, the CXCR7-binding capacities of modified CXCL12 were less affected. Furthermore, enzymatic processing reduced the lymphocyte and monocyte chemotactic activity of CXCL12 and severely impaired the antiviral activity against the HIV-1 strains NL4.3 and HE. Finally, co-expression of the substrate and enzyme was demonstrated in Crohn's disease. Therefore, we identified a new physiological down-regulator of CXCL12 function.

MIGRATING INTESTINAL DENDRITIC CELLS AND INTESTINAL IMMUNE RESPONSES

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Dendritic cells (DC) migrate continually from the intestine to the mesenteric nodes. We collect DC from rat thoracic duct lymph following mesenteric lymphadenectomy. In the steady state 3 DC subsets, differing phenotypically and functionally, migrate. These DC are powerful simulators of naïve T cells and do not bias towards a Th2 response. Inflammatory stimuli alter DC migration from the intestine and within the nodes. These changes are associated with release of TNFα and Type 1 interferons from plasmacytoid DC. Ovalbumin given to rats with indomethacin-induced intestinal inflammation stimulates CD4+ T cell activation and systemic IgG responses.

EFFECTS OF PI3K SIGNALING DRIVING LEUKOCYTE MIGRATION

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NOT AVAILABLE

Role of myeloid cells and innate immunity in cancer metastasis

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It has been well established that chronic inflammation functions as a potent tumor promoter that accelerates the emergence of many solid malignancies, such as gastric, liver and colorectal cancers. Previous studies have established important roles for inflammatory cytokines and transcription factors such as NF-kappaB and STAT3 in mediating the effects of inflammation on tumor development. Recently we have begun to investigate the role of inflammation and immune cells in metastatic progression, the most lethal complication in cancer. We found that metastatic progression in prostate cancer depends on a complex interplay between malignant epithelial cells and immune/lymphoid cells that are recruited into the growing tumors by factors produced by the malignant cells. Activation of IKKalpha-dependent NF-kappaB in immune/lymphoid cells in response to tumor stress results in the production of metastasis enhancing cytokines that act via IKKalpha in the malignant cell to suppress the expression of metastasis inhibitors.
In addition, we found that metastatic lung cancer cells secrete factors that act via Toll like receptors 2 and 6 to stimulate the production of TNF-alpha by tumor associated macrophages. TNF-alpha in turn stimulates the metastatic progression of lung carcinomas. In addition to illuminating the role of the inflammatory microenvironment in metastatic progression, these findings provide ample opportunities for the development of new therapeutic strategies.

POLARIZED INFLAMMATION AND TUMOR PROGRESSION

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Clinical and experimental evidence have highlighted that a major leukocyte population mainly present in the hypoxic areas of tumours, the so called tumour-associated macrophages (TAM), are the principal component of the leukocyte infiltrate supporting tumour growth. Evidence is accumulating for a 'switch' in macrophage phenotypes during the course of tumour progression. Whereas the functions of classically activated, 'M1' macrophages during chronic inflammation appear to predispose a given tissue to tumour initiation, in established tumours macrophages exhibit mainly the alternatively activated, 'M2' phenotype and are engaged in immunosuppression and the promotion of tumour angiogenesis and metastasis. Here I will discuss regulatory mechanisms driving the functional plasticity of macrophages during the course of tumour development, along with their implication for anti-cancer therapies aimed at prompting TAM to mount an effective antitumor response.

ABSTRACTS OF SELECTED AND POSTER PRESENTATIONS

P.001

CD137 INDUCES PROLIFERATION OF MURINE HEMATOPOIETIC PROGENITOR CELLS AND DIFFERENTIATION TO MACROPHAGES

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CD137 is a member of the TNF receptor family and reverse signaling through CD137 ligand, which is expressed as a cell surface transmembrane protein costimulates or activates antigen presenting cells. Activation of bone marrow cells through CD137 ligand induces proliferation, colony formation and an increase in cell numbers. Compared to total bone marrow cells the small subpopulation of primitive progenitor cells which expresses no lineage markers but expresses CD117 (lin-, CD117+ cells) responds with the same activities to CD137 ligand signaling, but at a significantly enhanced rate. Concomitantly to proliferation, the cells differentiate to colony-forming units granulocyte-macrophage, and then to monocytes and macrophages but not to granulocytes or dendritic cells. Hematopoietic progenitor cells differentiated in the presence of CD137 protein display enhanced phagocytic activity, secrete high levels of IL-10 but little IL-12 in response to LPS, and are incapable of stimulating T cell proliferation. This data demonstrates that reverse CD137 ligand signaling takes place in hematopoietic progenitor cells, in which it induces proliferation, an increase in cell numbers, colony formation and differentiation towards monocytes and macrophages.

P.002

INDUCTION OF PROLIFERATION AND MONOCYTIC DIFFERENTIATION OF HUMAN CD34+ CELLS BY CD137 LIGAND SIGNALING

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CD137 is a member of the TNF receptor family, and is involved in the regulation of activation, proliferation, differentiation and cell death of leukocytes. Bidirectional signaling exists for the CD137 receptor/ligand system as CD137 ligand which is expressed as a transmembrane protein, can also transduce signals into the cells it is expressed on. We have identified expression of CD137 in human bone marrow and expression of CD137 ligand on a subset of CD34+ cells. Crosslinking of CD137 ligand on CD34+ cells by CD137 ligand agonists induces activation, prolongation of survival, proliferation and colony formation. CD137 ligand agonists induce differentiation of early hematopoietic progenitor cells to colony-forming units granulocyte-macrophage, and subsequently to monocytes and macrophages but not to dendritic cells. These data uncover a novel function of CD137 and CD137 ligand by showing their participation in the growth and differentiation of hematopoietic progenitor cells.

P.003

EFFECTS OF TYPE I AND TYPE II INTERFERONS ON NEOPTERIN AND AMINO ACID METABOLISM IN HUMAN ASTROCYTE-DERIVED CELL LINES

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Neopterin is a pteridine preferentially produced by interferon (IFN)-γ stimulated human monocytic cells and a marker of immune activation during viral infections and inflammation. Increased concentrations of neopterin are observed in the peripheral blood of patients with infectious of inflammatory diseases. During several disorders of the central nervous system (CNS), neurological inflammatory processes and/or infections were found to be associated with increased neopterin concentrations in the cerebrospinal fluid (CSF). Infection by the human immunodeficiency virus type 1 (HIV-1) is accompanied by increased neopterin levels in plasma and CSF which correlate with the development of the acquired immunodeficiency syndrome (AIDS)-associated dementia. It is unclear, which cells are responsible for producing neopterin in the CNS. This study investigated whether IFN-α/β, IFN-γ or HIV-1 induce neopterin production in U87MG human astroglioma cells and in cells transfected with the HIV-1 receptor CD4 and coreceptors CXCR4 and CCR5. Results were compared to the induction of tryptophan degradation by indoleamine 2,3-dioxygenase (IDO).) and IFN-γ (1 µg/ml) significantlyβ + 10000 U/ml IFN-αIFN-α/β (1000 U/ml IFN- increased neopterin concentrations after 24 and 48 hours compared to untreated cells. The direct exposure of astrocytes to HIV did not affect neopterin production. Interestingly, IFN-γ but not IFN-α/β increased expression and activity of IDO. The amount of kynurenine, the bioproduct of IDO-mediated tryptophan-catabolism, was significantly increased after 12, 24 and 48 hours in supernatants of IFN-γ-treated cells compared to both control and IFN-α/β treated cells. Tryptophan concentration was reduced by 38 ± 7% in unstimulated control astrocytes after 48 hours culture compared to baseline tryptophan content of the culture medium used. IFN-γ further reduced tryptophan in the supernatants compared to control, consistent with an induction of IDO by the cytokine. Surprisingly tryptophan concentration in the supernatant of IFN-α/β-treated astrocytes was higher compared to control cultures, suggesting a general inhibitory effect of IFN-α/β on amino acid uptake or protein synthesis. When these changes were compared to other aromatic amino acids, also the concentrations of phenylalanine and tyrosine were reduced by 33 ± 2% and 20 ± 4% in control astrocytes after 48 hours, and again concentrations of these amino acids were higher upon treatment with IFN-α/β. Together with the reduced consumption of tryptophan, this observation may relate to the antiproliferative activity of type I interferons. The induction of neopterin formation by both IFN-α/β and IFN-γ is a characteristic that astrocytes share with monocyte-derived dendritic cells, which also showed similar responsiveness to IFN-α/β and IFN-γ.

P.004

The role of HIF-prolyl hydroxylase 2 (PHD-2) during inflammation and tumor development in mice

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It has been well described that areas of inflamed and injured tissues expose a high concentration of inflammatory mediators as well as low levels of oxygen (= hypoxia) (e.g. joints during arthritis, local bacterial infection or rapid tumor growth). In order to successfully fight an infection, different molecular mechanisms help cells of the innate immune response to adapt and overcome hypoxia, and function under these severe conditions. The master switch for the transcriptional activation under deprived oxygen concentrations are the hypoxia inducible factors (HIFs), and it is estimated that they regulate about 2% of all genes, including those involved in angiogenesis, metabolism, and cell survival/apoptosis. Only recently their post-translational regulators, the HIF-prolyl hydroxylases (PHDs), have been identified. To date, little is known about the exact function of these enzymes in vivo, and for that reason our new research group focuses on understanding their function in monocytes/macrophages during different pathological and physiological processes in mice. For that purpose, we developed a PHD-2 conditional deficient mouse line that we are using in combination with a self-generated macrophage-specific Cre line. The resulting knock-out mice are being used in models related to tumor development and metastasis, as well as in local inflammatory disorders, with an explicit focus on the cross-talk between the macrophages and the surrounding tissue (e.g. skin, endothelium or tumor cells). These studies will hopefully lead to a better understanding of the interplay between the macrophages and their relation to oxygen homeostasis, and may therefore be helpful in the development of new therapeutic strategies.

P.005

Identification of discrete tumor-induced myeloid-derived suppressor cell subpopulations with distinct T-cell suppressive activity

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The induction of CD11b+Gr-1+ myeloid-derived suppressor cells (MDSCs) is an important immune-evading mechanism employed by tumors. However, the exact nature and function of MDSCs remain elusive, especially because they constitute a heterogeneous population which has not yet been clearly defined. Here, we identified two distinct MDSC subfractions with clear morphologic, molecular and functional differences. These fractions consisted of either mononuclear cells (MO-MDSCs), resembling inflammatory monocytes, or low-density polymorphonuclear cells (PMN-MDSCs), akin to immature neutrophils. Interestingly, both MO-MDSCs and PMN-MDSCs suppressed antigen-specific T-cell responses, albeit by employing or disrupting distinct effector molecules and signaling pathways. Blocking IFN- STAT1 partially impaired suppression by MO-MDSCs, for which nitric oxide (NO) was strictly required for the was one of the mediators. In contrast, while IFN- suppressor function of PMN-MDSCs, this did not rely on STAT1 signaling nor NO production. Finally, MO-MDSCs were shown to be potential precursors of highly anti-proliferative NO-producing mature macrophages. However, distinct tumors, irrespective of host genetic background, differentially regulated this inherent MO-MDSC differentiation program, indicating that this was a tumor-driven phenomenon. Overall, our data refine the tumor-induced MDSC concept by uncovering mechanistically distinct MDSC subfractions, potentially relevant for MDSC-targeted therapies.

P.006

COORDINATED REGULATION OF MS4A GENES DURING MACROPHAGE ALTERNATIVE ACTIVATION

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Macrophages are first-line cells of the innate immune system that provide immediate defence against foreign agents, contribute to inflammation resolution and tissue repair, and assist during the induction of the adaptive immune response. These complex and divergent functions largely depends on the influence and Th2 cytokines of the extracellular milieu, with Th1 cytokines (IFN (IL-4/IL-13) eliciting the so-called polarized classical (M1) or alternative (M2) activation phenotypes, respectively. A comprehensive analysis of the gene expression profiles associated with human macrophage polarization led to the identification of different transcriptional signatures. Among genes associated with macrophage alternative activation, a coordinated regulation of MS4A family RIβ, and other 10 members was noticed. The MS4A gene family includes CD20, Fc poorly defined members, all encoding structurally-related cell-surface proteins spanning the membrane four times. Among these genes, our transcriptional profile analysis highlighted a coordinated upregulation of the MS4A4A, MS4A6A, and MS4A7 genes, while other MS4A family members where either not expressed or not regulated in macrophages. These genes are encoded by a gene cluster located on 11q12, a chromosomal location previously associated with increased susceptibility to allergy, atopy and asthma. Our analysis of MS4A4A, MS4A6A, and MS4A7 expression revealed that they are restricted to myeloid cells (macrophage and monocyte-derived dendritic cell), and are upregulated during macrophage alternative activation induced by different polarizing mediators, including IL-4, IL-10, and glucocorticoids. Although also supporting macrophage alternative activation, TGF-β was inactive. Finally, EGFP-tagged MS4A4A, MS4A6A, and MS4A7 expressed in CHO cells showed that all molecules traffic to the cell membrane. Though the biological functions of these MS4A proteins has not jet been defined, their membrane localization and the structural relationship with other better characterized MS4A members suggest a potential involvement in signal transduction, either as components of multimeric receptor complexes or as components of ligand-gated ion channels.

P.007

DIFFERENTIAL RESPONSE TO ACTIVATION SIGNALS OF MONOCYTE-DERIVED DENDRITIC CELLS

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CD14high monocytes cultured in the presence of GM-CSF and IL-4 differentiate to CD14-CD1a+ dendritic cells by consecutive steps through CD14lowCD1a- cells. The ratio of CD1a- and CD1a+ cells derived from monocytes (moDC) of various donors showed high variability pointing to individual factors regulating moDC differentiation and CD1a dichotomy. One of these modulatory factors was identified as the lipoprotein-mediated activation of PPARg that screws moDC development to the CD14lowCD1a-PPARγ+ phenotype characterized by high internalizing capacity and the potential to activate iNKT cells (Gogolak et al. 2007). We also observed that the transition of CD1a- cells to more mature CD1a+ moDC could be blocked by various activation stimuli suggesting the different maturation states of these subsets. To assess the response of these cell types to further stimulation we sorted the CD1a- and CD1a+ moDC subsets on day 5 and studied their response to CD40 ligand, polyI:C, TLR7/8 ligand or LPS by monitoring phenotypic changes and cytokine production in fresh medium. Cell surface expression of CD83 and CCR7 was significantly higher in CD1a+ cells than in their CD1a- counterparts in line with their elevated production of pro-inflammatory cytokines and IL-12p70 secretion. Stimulation by LPS however, required DC-conditioned medium for their subtype-specific activation and the early exposure of DC to LPS blocked further responses. These findings prompted us to search for regulatory pathways modulating LPS-induced activation of moDC subsets. We showed that the response of moDCs to LPS stimulation is highly dose and timing dependent and soluble CD14 released from the differentiating cells has a modulatory effect on the LPS response. We also found that some of the microRNAs (miR) shown to be involved in the regulation of the NF-κB pathway are differentially expressed in these subsets and miR146a and miR155 are involved in controlling the migration and the LPS response of moDCs. As CD14+CD1-PPARg+ and CD14-CD1a+PPARg- moDCs are detectable in peripheral lymphoid tissues and also in the vicinity of epithelial surfaces, these results may have an important impact on the collaboration of DC subsets in priming inflammatory or tolerogenic immune responses.

P.008

A NOVEL ROLE OF DENDRITIC CELLS IN THE PROTEIN C PATHWAY IN INFLAMMATORY BOWEL DISEASE: THE MISSING LINK BETWEEN COAGULATION AND INFLAMMATION.

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Activated Protein C (aPC) is a natural anticoagulant, that displays potent anti-inflammatory and cytoprotective activities. Protein C (PC) circulates in the plasma as a zymogen and is activated by thrombomodulin (TM) and the endothelial protein C receptor (EPCR). The anti-inflammatory activities of aPC are mediated by its interaction with EPCR and TM and the subsequent cleavage of the protease-activated receptor-1 (PAR-1) on endothelial cells. Although the anticoagulant effects of APC are well known, its anti-inflammatory properties are less understood. The aim of this study was to investigate the involvement of DC in the PC pathway in the intestine. By cytofluorimetric and Real Time analysis it was found that monocyte-derived immature DC express significant levels of EPCR, TM and PAR-1. Inflammatory stimuli, such as, LPS, TNF-a and CD40L, which induce DC maturation, lead to a dramatic downregulation of the expression of these receptors. In contrast, IL-10 induced up-regulation of the EPCR-TM-PAR-1 complex by DC. Immunohistochemical staining of colonic sections of normal and actively inflamed Inflammatory Bowel Disease (IBD) patients showed strong immunoreactivity for EPCR and TM in the colonic mucosa and submucosa of normal subjects which colocalizes with DC-like cells. On the contrary, IBD mucosa expressed reduced levels of both receptors.

These results show for the first time that DC express the PC pathway machinery that can be modulated during maturation, and suggest that DC may bridge inflammation and coagulation. In addition in chronic inflammatory diseases such as IBD PC pathway expression by intestinal DC is impaired, and restoring such pathway may have a therapeutic relevance.

P.009

LIGAND-DEPENDENT OPTIMIZATION OF CHEMOKINE DECOY RECEPTOR D6 SCAVENGER PERFORMANCE: A NEW ADAPTIVE MECHANISM ALLOWING D6 TO COPE WITH TISSUE INFLAMMATION

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The decoy receptor D6 plays a non-redundant role in the control of inflammatory processes through scavenging of inflammatory chemokines. However it remains unclear how it is regulated. Here we show that D6 scavenging activity relies on unique trafficking properties. Under resting conditions, D6 constitutively recycled through both a rapid wortmannin (WM)-sensitive and a slower brefeldin A (BFA)-sensitive pathway maintaining low levels of surface expression that required both Rab4 and Rab11 activities. In contrast to "conventional" chemokine receptors that are downregulated by cognate ligands, chemokine engagement induced a dose-dependent BFA-sensitive Rab11-dependent D6 redistribution to the cell membrane and a corresponding increase in chemokine degradation rate. Thus, the energy-expensive constitutive D6 cycling through Rab11 vesicles allows a rapid, ligand concentration-dependent, increase of chemokine scavenging activity by means of receptor redistribution to the plasma membrane. Prelimary data also demonstrated that D6 carboxy-terminus end is essential to support its ligand-dependent upregulation on cell surface. D6 is not regulated at a transcriptional level in a variety of cellular contexts, thus ligand-dependent optimization of its scavenger performance represents a rapid and unique mechanism allowing D6 to control inflammation.

P.010

CHEMOKINE DEGRADATION BY LOCAL EXPRESSION OF THE CHEMOKINE DECOY RECEPTOR D6 INHIBITS TUMOR GROWTH

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The chemokine decoy receptor D6 binds most of the inflammatory CC chemokines and it is extremely efficient in targeting chemokines to degradation. In vivo models with D6-/- mice have highlighted its important role in the resolution of inflammation and in the protection from inflammation-driven carcinogenesis. D6 is expressed at high levels by lymphatic vessels endothelium and its expression was reported also by some vascular tumors. Here we report that D6 is also expressed by HHV8-infected spindle cells of Kaposi's Sarcoma (KS) lesions. D6 expression is higher in primary lesions while it is expressed at lower levels by nodular lesions. To address D6 role in KS biology, KS-Imm human Kaposi's sarcoma cells overexpressing D6-EGFP chimeric protein were studied in vitro and in vivo. In vitro, D6 expression did not influence cell proliferation while when KS-Imm/D6 cells were subcutaneously injected in the flank of CD-1 nude mice showed significantly reduced growth compared to mock transfected cells. The amount of murine and human CCL2 extracted from D6-expressing tumors was significantly reduced. Moreover FACS and immunohistochemistry analysis showed that these tumors had limited leukocyte infiltration. These data suggest that D6, through its scavenging activity, regulate the bioavailability of inflammatory chemokines and therefore controls leukocyte accumulation at tumor site that support tumor growth and angiogenesis.

P.011

SUBCAPSULAR SINUS MACROPHAGES IN LYMPH NODES CLEAR LYMPH-BORNE VIRUSES AND PRESENT THEM TO ANTIVIRAL B CELLS

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Lymph nodes (LNs) prevent systemic dissemination of pathogens, such as viruses that enter the body's surfaces, from peripheral sites of infection. They are also the staging ground of adaptive immune responses to pathogen-derived antigens. It is unclear how virus particles are cleared from afferent lymph and presented to cognate B cells to induce antibody responses. Here, we identify a population of CD11b+CD169+MHCII+ macrophages on the floor of the subcapsular sinus (SCS) and in the medulla of LNs that capture viral particles within minutes after subcutaneous (sc) injection. SCS macrophages translocated surface-bound viral particles across the SCS floor and presented them to migrating B cells in the underlying follicles. Selective depletion of these macrophages compromised local viral retention, exacerbated viremia of the host, and impaired local B cell activation. These findings indicate that CD169+ macrophages have a dual physiological function. They act as innate 'flypaper' by preventing the systemic spread of lymph-borne pathogens and as critical gatekeepers at the lymph-tissue interface that facilitate B cell recognition of particulate antigens and initiate humoral immune responses.

P.012

APOPTOTIC CELLS STIMULATE PPARgamma THEREBY TRANSREPRESSING NFkappaB

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Phagocytosis of apoptotic cells (AC) avoids the release of harmful cell contents of dying cells and provokes an anti-inflammatory phenotype switch in macrophages. The altered phenotype is characterized by an increased anti-inflammatory cytokine production, but a reduced release of pro-inflammatory mediators. Mechanisms explaining diminished pro-inflammatory cytokine formation remain elusive. We provide evidence that peroxisome proliferator-activated receptor γ (PPARγ) is activated in response to AC, thus contributing to the anti-inflammatory phenotype. Interaction of AC with RAW264.7 macrophages attenuated LPS-induced NFκB activation and concomitant target gene expression of e.g. TNFα and IL-6 compared to controls. Pretreatment of macrophages with the PPARγ antagonist GW9662 prior to AC and LPS reversed NFκB inhibition. Moreover, exposing macrophages, which overexpress a dominant/negative (d/n) PPARγ mutant, to AC restored LPS-induced NFκB activation and concomitant target gene expression. Because expression of a PPARγ-Δaa32-250 deletion mutant failed to restore NFκB inhibition in response to AC, we analyzed protein motives located in this PPARγ domain. We noticed a potential sumoylation site at K77 and reasoned that sumoylation of PPARγ might account for NFκB inhibition. In line with our assumption, the mutation of K77 abolished the ability of AC to inhibit NFκB transactivation. Moreover, ChIP analysis demonstrated that AC prevent LPS-induced removal of the nuclear receptor co-repressor (NCoR)-histone deacetylase-3 (HDAC3) complex from the κB site within the TNFα promoter in RAW264.7 macrophages. This was not seen in macrophages overexpressing the d/n PPARγ mutant. We conclude that sumoylation of PPARγ in response to AC prevents clearance of NCoR, thereby inhibiting NFκB-dependent target gene expression. These data provide a mechanism, how AC provoke attenuated pro-inflammatory cytokine production in macrophages.

P.013

APOPTOTIC CELL-DERIVED S1P PROMOTES CREB-MEDIATED ARGINASE II EXPRESSION

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Phagocytosis of apoptotic cells (AC) by macrophages (MΦ) alters their phenotype towards alternative activation. Polarized MΦ are characterized by attenuated proinflammatory cytokine expression as well as nitric oxide (NO) production. Recently, we demonstrated that impaired NO production in response to AC or AC-conditioned medium (CM), was due to arginase ll (ARG ll) up-regulation, which competes with the inducible NO synthase (iNOS) for the common substrate L-arginine. Here, we aimed at elucidating the signaling pathway that provokes CM-mediated ARG ll up-regulation in MΦ. The use of a luciferase reporter plasmid containing a small fragment of the ARG ll-promoter (-262bp to -1bp) pointed to the involvement of the transcription factor c-AMP responsive element binding protein (CREB). This assumption was confirmed by EMSA analysis showing that CM provoked CREB-binding and by using decoy oligonucleotides, which scavenged CREB, thereby preventing it from binding to its target genes, and thus ARG ll expression. Inhibition of ERK5 by UO126 suggested this mitogen activated protein kinase (MAPK) pathway to be involved in CM-mediated CREB activation (shown by EMSA analysis) as well as ARG ll induction. Finally, we prepared CM with a depletion of sphingolipids by using the sphingomyelinase inhibitor GW4869, the ceramidase inhibitor NOE or by employing CM from apoptotic MCF-7 cells, where sphingosine kinase 2 (Sk2) had been knocked-down, thus producing less sphingosine-1-phosphate (S1P). The ability of the modified CMs to induce ARG ll was impaired compared to CM from wild-type apoptotic MCF-7 cells, thus suggesting that S1P is important for ARG ll up-regulation in MΦ. We conclude that AC release S1P, which provokes activation of the ERK5 signaling cascade, leading to CREB phosphorylation/activation and concomitant ARG ll expression.

P.014

LIPOLYSACCHARIDES FROM BORDETELLA PERTUSSIS AND BORDETELLA PARAPERTUSSIS DIFFERENTLY MODULATE HUMAN DENDRITIC CELL FUNCTIONS RESULTING IN DIVERGENT PREVALENCE OF TH-17 POLARIZED RESPONSES

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Bordetella pertussis and parapertussis are the etiological agents of pertussis, yet the former has a higher incidence and is the cause of a more severe disease, in part due to pertussis toxin. To identify other factors contributing to the different pathogenicity of the two species, we analyzed the capacity of structurally different lipooligosaccharide from B. pertussis (BpLOS) and lipopolysaccharide from B. parapertussis (BppLPS) to influence immune functions regulated by dendritic cells (DC). Either BpLOS and BppLPS triggered TLR4 signaling and induced phenotypic maturation and IL-10, IL-12p40, IL-23, IL-6, IL-1beta production in human monocyte-derived DC (MDDC). BppLPS was a stronger inducer of all these activities as compared to BpLOS, with the notable exception of IL-1beta, which was equally produced. Only BppLPS was able to induce IL-27 expression. In addition, while MDDC activation induced by BppLPS was greatly dependent on soluble CD14, BpLOS activity was CD14-independent. The analysis of the intracellular pathways showed that BppLPS and BpLOS equally induced IkappaB-alpha and p38 MAPK phosphorylation, but BpLOS triggered ERK1/2 phosphorylation more rapidly and at higher levels than BppLPS. Furthermore, BpLOS was unable to induce MyD88-independent gene induction, which was instead activated by BppLPS, witnessed by STAT1 phosphorylation and induction of the interferon dependent genes IRF-1 and IP-10. These differences resulted in a divergent regulation of Th responses, BpLOS-MDDC driving a predominant Th-17 polarization. Overall, the data observed reflect the different structure of the two LPSs and the higher Th-17 response induced by BpLOS may contribute to the severity of pertussis in humans.

P.015

CD11b-LOW MACROPHAGES EXERT PRO-RESOLVING PROPERTIES MODULATED BY RESOLVINS AND GLUCOCORTICOIDS DURING THE RESOLUTION OF INFLAMMATION

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During the resolution phase of inflammation the corpses of apoptotic leukocytes are cleared by macrophages in a nonphlogistic fashion that results in diminished responses to bacterial moieties and production of anti-inflammatory cytokines. Complement receptor 3 (CR3) and pro-resolving lipid mediators promote the engulfment of apoptotic leukocytes by macrophages. Here, we present evidence for the emergence of pro-resolving, CD11b-low macrophages in vivo during the resolution of murine peritonitis, and their distinct properties in terms of apoptotic leukocyte engulfment and responses to LPS. In addition, we found the pro-resolving lipid mediators resolvin (Rv) E1 and RvD1, as well as the glucocorticoid dexamethasone (Dex), to regulate pro-resolving macrophage functions in vivo. In fact, resolvins and Dex exhibited a novel pro-resolving function by reducing the apoptotic leukocyte ingestion requirement for CD11blow macrophage generation. Thus, we suggest that the emergence of pro-resolving CD11b-low macrophages is an essential component in the termination of acute inflammation.

P.016

Presence of local precursors for dendritic cells in the pancreas of fetal and neonatal mice

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Under steady state conditions low levels of dendritic cells (DC) are present in the pancreas. The accumulation of DC in the pre-diabetic pancreas is a hallmark of the development of autoimmune diabetes. Previous studies of our group suggested that an influx of blood monocytes cannot form the basis for this accumulation. Therefore we hypothesized that the pancreas must contain local precursors for DC, from which the accumulation of DC in the pre-diabetic pancreas occurs. Here we study the existence of such local precursors in the fetal and neonatal pancreas of mice. Cells from fetal (E15.5) pancreases of C57BL/6 mice were isolated followed by flowcytometric analysis using ER-MP58, Ly6C, CD31 and CD115 as markers for myeloid DC precursors and CD11c for differentiated DC. Also E15.5 pancreases were cultured for 5 days in the presence of GM-CSF followed by flowcytometric analysis of the migrated cells to investigate DC development from precursors. In addition, ER-MP58+ cells sorted from fetal pancreases were cultured with GM-CSF to study the generation of DC. Low numbers of CD11c+ cells were present in the fetal pancreas as well as myeloid progenitors expressing ER-MP58 carrying several markers of common myeloid precursors (Ly6C, CD31 and CD115). The pancreatic explant cultures revealed a significant increase in the CD11c+ population due to culture with GM-CSF. Sort experiments of the ER-MP58+ cells showed that these cells were precursors for CD11c+ DC with co-expressing MHC II and CD86. To investigate the presence of proliferating precursors after birth, pancreases of neonatal C57BL/6 mice (1 day to 5 weeks) were isolated followed by immunohistochemical staining using ER-MP58 combined with the proliferation marker Ki67. In neonatal pancreas ER-MP58+ cells were still present decreasing with age. Proliferation of the precursors was detected from 1 day to 5 weeks of age, with the strongest proliferation at 1 week. In conclusion, ER-MP58+ cells already present in the pancreas of fetal mice of E15.5 serve as local precursors for pancreas DC.

P.017

Divergence in toll-like receptor-4 responses between primary human and mouse macrophages

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The ability to mount an effective innate immune response is clearly under strong evolutionary selection pressure from pathogen challenge. Pathogens evolve rapidly to evade host defence, and mammals must possess an array of alternative effector mechanisms to respond to different pathogens. The evolution of coding sequences has been well characterised, however, wide-scale evolutionary analysis of promoter sequences has not been possible, as accurate transcription starts (and thus promoter positions) were unavailable. We hypothesized that evolution in gene regulatory sequences, allowing for variation in gene expression, contributes to divergence in innate immune responses between mouse and human. Indeed, polymorphisms in gene regulatory sequences have already been shown to drive divergent innate immune responses in Drosophila. We used microarrays to compare the transcriptional responses of primary human and mouse macrophages to the archetypal inflammatory stimulus, lipopolysaccharide (LPS) that signals via toll-like receptor-4 (TLR4). Although a large cluster of genes was similarly regulated, there was also considerable divergence in gene expression between the species. The divergent regulation of many genes with key roles in innate immunity was validated, including members of the TLR signalling pathway (e.g. Cd14), transcription factors (e.g. Stat4), cytokines (e.g. Ccl20) and chemokines (e.g. Cxcl13). Macrophage promoters were defined by genome-wide identification of transcription start sites by deep CAGE (Cap Analysis of Gene Expression) in both species. The impact of promoter evolution on transcriptional regulation on a genome-wide scale is a focus of current investigation. This study provides the first systematic comparison of mouse and human gene expression in innate immunity, and gives insight into immune system evolution and the limits of mice as model organisms for human infection and inflammatory disease.

P.018

A METHOD FOR THE ISOLATION AND PURIFICATION OF GRANULOMA MACROPHAGES FROM THE LIVERS OF BCG-INFECTED MICE

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BCG infection in mice induces granulomatous inflammation in liver, lung and spleen. Granulomas are characterised by focal accumulations of activated macrophages (Mf), transformed into epithelium-like cells, and surrounded mainly by T cells, thus containing the infection. Studies on gene expression during M. tuberculosis or BCG infection always use in vitro differentiated and infected Mf, or whole tissue lysates. However, in vitro conditions poorly reflect in vivo situations, and whole tissue gene expression profiling includes the risk of missing interesting genes and of detecting irrelevant genes. We therefore set-up a protocol to isolate and purify granuloma Mf from livers of BCG-infected mice to perform gene expression studies on Mf which are differentiated in vivo.
Granuloma Mf are derived three weeks after infection, when the Mf are fully developed and the granulomas reach their largest size. The liver is perfused with PBS via the hepatic portal vein, and the tissue is passed through a 190 µm stainless sieve which does not disrupt the granulomas. To enrich the granulomas, they are allowed to sediment by gravity, and the final pellet is digested using collagenase type IV. The digested material is passed through a cell strainer (70 µm) and loaded on Histopaque 1119 for density gradient centrifugation. The interphase contains the mononuclear cell fraction. The granuloma Mf are further purified using indirect Magnetic Cell Sorting (MACS) with a combination of anti-F4/80-biotin antibodies and anti-biotin microbeads. Viability of the resulting cell population is checked by trypan blue exclusion. The purity of the cell fraction is determined by surface antigen expression (FACS) and morphology (cytospin). Using this procedure, we were able to purify granuloma Mf with > 90 % purity. These cells can be used for several applications, including gene expression analysis.

P.019

PLASMACYTOID DENDRITIC CELLS IN MULTIPLE SCLEROSIS: INTRACEREBRAL RECRUITMENT AND IMPAIRED MATURATION IN RESPONSE TO INTERFERON-β.

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The involvement of plasmacytoid DC (pDC) in multiple sclerosis (MS), a population of DC implicated in several autoimmune diseases, are still poorly understood. pDC accumulation was observed in white matter lesions and meninges of MS brain where abundant expression of the type I IFN-induced protein MxA, mainly in perivascular CD3+ lymphocytes, indicated type I IFN production from activated pDC. The pDC chemoattractant chemerin was detected in intralesional cerebrovascular endothelia while the chemerin receptor was expressed on infiltrating leukocytes, including pDC. Because these results suggested active recruitment and maturation of pDC in the inflamed brain, we asked whether IFN-β therapy had an impact on pDC phenotype and function. IFN-β, the first drug approved for the treatment of MS, is thought to attenuate the disease-associated inflammatory process by acting on several leukocyte populations. Thus, the effect of IFN-β therapy on pDC phenotype and function was evaluated in MS patients before and during IFN-β treatment. Ex-vivo analysis of pDC from the blood of MS patients before and during IFN-β treatment revealed that IFN-β did not modify the frequency and immature phenotype of circulating pDC but caused a reduction of the cell-surface expression of MHC class II and BDCA2 molecules and the concomitant induction of two IFN-inducible markers, CD38 and B7H1. These findings indicated that exposure of circulating and, possibly, brain-infiltrating pDC to IFN-β in vivo might result in reduced Ag-presenting and T-cell stimulatory capacity while the absence of an effect on ChemR23 expression indicates that the ability of pDC to migrate in response to chemerin may not be affected by IFN-β treatment. In parallel, through induction of the inhibitory molecule B7H1, IFN-β could enhance the ability of pDC to downregulate pathogenic T cells. When pDC from MS patients treated with IFN-β were exposed in vitro to the activation stimulus CpG, a significant reduction of CD83 and CD86 expression was observed, indicating that IFN-β impairs pDC maturation. The effects of IFN-β on pDC maturation were further investigated in vitro using pDC isolated from healthy donors. We observed that pre-treatment of pDC with IFN-β reduced their maturation induced by different activating stimuli, resulting in impaired secretion of IFN-α and other pro-inflammatory cytokines and in reduced T-cell stimulatory ability. It can be envisaged that pDC homing to the inflamed CNS, after being exposed to IFN-β in the peripheral blood, poorly respond to the maturative stimuli encountered in the inflamed tissue and, in turn, might display a reduced ability to promote pathogenic humoral and cellular immune responses due to the lack of an appropriate mature phenotype.

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P.020

INCREASED TNF EXPRESSION IN CD43++ MURINE BLOOD MONOCYTES

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To study blood monocyte populations in mouse we have used the MacGreen mouse model, which expresses the green-fluorescent protein (GFP) under the control of promoter of the murine M-CSF receptor (CSF1 receptor, c-fms). Since both monocytes and granulocytes show GFP expression in this model the latter cells were excluded by staining with the Ly6G granulocyte marker. GFP+ Ly6G- blood monocytes were found to account for an average of 246 ± 121 cells/µl in these mice. These monocytes can be subdivided into CD43+ GR-1+ cells and CD43++ GR-1- cells, with the latter cells accounting for 140 ± 77 cells/µl, i.e. about 60% of all blood monocytes. After intraperitoneal injection of lipopolysaccharide (LPS) both blood monocyte subpopulations were depleted. The same was true after intranasal infection with Streptococcus pneumoniae but here the CD43++ subpopulation was preferentially reduced to 4 cells/µl.

For the study of TNF expression cells were stimulated in-vitro with LPS from S. abortus equi followed by intracellular staining and analysis in flow cytometry. Over a dose range of 10 to 100 ng LPS/ml, TNF protein production was significantly higher in the CD43++ monocyte subset. At 1000 ng LPS/ml 90% of all CD43++ monocytes stained positive for TNF and in terms of fluorescence intensity TNF was 5-fold higher compared to the CD43+ monocytes.

These data indicate that the murine CD43++ monocyte subset exhibits features of pro-inflammatory monocytes and is functionally homologeous to the human CD14+CD16+ monocytes.

P.021

THE PROTOZOAN PARASITE *THEILERIA ANNULATA* ALTERS THE DIFFERENTIATION STATE OF THE HOST MACROPHAGE

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The schizont stage of the tick-borne, apicomplexan parasite *Theileria annulata* predominantly resides within bovine macrophages. The parasite induces host cell transformation by a largely unknown mechanism, which does not involve the integration of parasite DNA into the host cell genome. As a result the transformation event is completely reversible and requires the presence of viable parasite. *T. annulata* infection is associated with loss of characteristic macrophage functions and phenotypic markers, e.g. impaired phagocytosis, reduced production of nitric oxide, superoxide and pro-inflammatory cytokines in response to stimulation and down-regulation of CD14 and CD11b. Therefore, it has been postulated that *T. annulata* induces the macrophage to revert back to a de-differentiated state. However, *T. annulata* infected macrophages exhibit enhanced antigen presenting capabilities and induce naïve T lymphocyte activation and proliferation and therefore do not resemble typical monocytes or myeloid progenitor cells.

To investigate the differentiation state of *T. annulata* infected cells, the mRNA levels of the MAF transcription factors c-maf and mafB were measured during *T. annulata* infection. c-maf and mafB play important roles in the regulation of macrophage differentiation. Furthermore, c-maf is a marker of macrophage differentiation, with expression levels increasing from myeloid progenitor cells to macrophages. The levels of c-maf and especially mafB are significantly lower in *T. annulata* infected cell-lines than in bovine monocytes or monocyte-derived macrophages. Treatment of *T. annulata* infected cells with the theileriacidal drug buparvaquone induced the up-regulation of c-maf and mafB, which was correlated with the expression of down-stream transcriptional targets, e.g. integrin beta 7 and interleukin 12A. Furthermore, the analysis has been expanded by investigating the expression of other transcription factors involved in the regulation of monocyte/macrophage differentiation. Levels of several transcription factors, e.g. Pu.1 and AML1, increased upon buparvaquone treatment of *T. annulata* infected cell-lines.

Therefore, *T. annulata* suppresses the expression of several transcription factors involved in regulating the differentiation of monocytes and macrophages. These results support the hypothesis that *T. annulata* modulates the host macrophage differentiation state. This may be a major mechanism employed by *T. annulata* to survive within the infected macrophage; either by promoting host cell proliferation or by suppressing the appropriate bovine immune response that would eradicate the infection.

P.022

IDENTIFICATION OF A NOVEL DENDRITIC CELL TYPE FROM HUMAN LYMPHOID TISSUE EXPRESSING AIRE (AUTOIMMUNE REGULATOR)

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Self-reactive T-cells from the developing T-cell repertoire are eliminated in the thymus by central tolerance (CT) mechanisms, a process predominantly mediated by medullary epithelial cells expressing AIRE. Although remarkably efficient, few potentially autoreactive T-cells escape CT selection and reach the periphery, where peripheral tolerance (PT) induction is then required to prevent autoimmunity. Recent data (Lee et al., Nat Immunol 2007; 8(2):181-90) have shown that "stromal" cells in murine lymph nodes (LN) express AIRE and could play a role in the induction of PT. Using immunohistochemistry, we investigated AIRE expression in a large number of different human lymphoid and non-lymphoid tissues and characterized the phenotype of AIRE+ cells.

AIRE+ cells were exclusively identified in LN (with predilection of abdominal LN), tonsils and mucosal-associated lymphoid tissues. AIRE+ cells were rare and localized in the paracortical area, close to high endothelial venules. Double immunohistochemistry showed that AIRE+ cells consistently co-expressed membranous HLADR and fascin (that highlight their dendritic morphology), and about 50% of them were positive for S100 and DC-LAMP/CD208, while only a few AIRE+ cells also expressed CD11c and CD83. Antigens of the lymphoid/myeloid lineages (including CD45RB, CD45RA, CD45RO, CD34, CD163, CD68, CD123, CXXL13/BCA1), stromal and endothelial cells (smooth muscle actin, desmin, vimentin, CD31, Factor-VIII-RA), and cytokeratins 8/18/19 (CAM5.2) were negative. Cells with a similar phenotype were found in the thymic medulla. AIRE staining on cytospins obtained from magnetic beads cell sorting from mesenteric LNs confirmed the presence of a population of CD45-/HLA-DR+/AIRE+ cells. On FACS analysis the CD45- sorted cells comprise about 5% of the total cell population and HLA-DRhi+ cells represent about 2.3% of this CD45- fraction. Moreover, FACS analysis confirmed that about 25% of the CD45-/HLA-DR+/AIRE+ sorted cells express CD83. Taken together, these data show that human lymphoid tissues contain a novel rare subset of dendritic cells that express AIRE, partially show an activated phenotype (DC-LAMP+, HLA-DR+, CD83+) and are possibly involved in PT.

P.023

PLASTICITY IN MACROPHAGE POLARIZATION REGULATES P2X7 RECEPTOR ACTIVITY TO ENHANCE INFLAMMATORY RESOLUTION

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Macrophage polarization is emerging as a new paradigm in immune regulation, especially in the resolving phase of inflammation and its implication in chronic inflammatory diseases where the ability to achieve resolution is impaired. Macrophages polarize in response to cytokines produced by T-helper leukocytes and they can either initiate and amplify (M1 polarization state) or resolve (M2 polarization state) the inflammatory response. Extracellular ATP is found in high levels in inflamed or damaged areas and has been considered a danger signal in M1 macrophage by acting through P2X7 receptors (P2X7R), being one of the main physiological signals that leads to a rapid activation of caspase-1 and subsequent processing and release of the pro-inflammatory cytokine interleukin-1beta (IL-1beta). We have investigated the function of extracellular ATP and P2X7R during a macrophage polarization continuum protocol, where the macrophages were either polarized from M1 to M2 or from M2 to M1. During the polarization continuum we examined the expression of 20 pro- and anti-inflammatory genes by quantitative PCR, the morphology of the macrophages using high-resolution deconvolution microscopy and functional experiments for P2X7R induced IL-1beta release. Surprisingly we found that polarization of the macrophages towards M2 turns the action of extracellular ATP from an inflammatory signal in M1 macrophages to potent anti-inflammatory signal in M2 macrophages, thus helping the resolution of inflammation. Moreover, when P2X7Rs in M1 macrophage were blocked (by selective receptor antagonists or by using macrophage from P2X7R knockout mice), ATP was similarly anti-inflammatory. In both situations, the action of ATP was found not to involve any known purine receptor. Instead it was due to di and tri (but not mono) phosphates; these phosphates and pyrophosphate-derived drugs (bisphosphonates) potently inhibited caspase-1 activation induced by toxins or bacterial challenge. These findings open a new scope for the use of P2X7R selective antagonist in the treatment of chronic inflammatory diseases and also distinguish an unexpected mechanism for the anti-inflammatory actions of clinically used bisphosphonates.

P.024

SIGNAL-TRANSDUCING ADAPTOR PROTEIN 1 (STAP1) INTERACTS WITH THE M-CSF-RECEPTOR AND REGULATES MICROGLIA AND MACROPHAGE ACTIVATION

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Signal-transducing adaptor protein 1 (Stap1) has been initially cloned as a stem cell specific adaptor protein containing pleckstrin and Src homology 2 domains. In a genome-wide DNA-microarray study, we identified a strong overexpression of Stap1 in activated retinal microglia from retinoschisin-deficient mice. Quantitative RT-PCR in a murine tissue panel showed that Stap1 transcripts are constitutively expressed in microglia and tissue macrophages and that Stap1 levels are further inducible by TLR-ligands . Transient overexpression of a GFP-Stap1 fusion proteinγ(LPS and CpG) and IFN- in BV-2 microglia-like cells and RAW264.7 macrophages enhanced pro-inflammatory cytokine expression after LPS-stimulation. Immunocytochemistry and antibody pull-down assays revealed that GFP-Stap1 colocalizes and interacts with the M-CSF-receptor. In addition, BV-2 and RAW264.7 cells stably expressing GFP-Stap1 showed reduced basal and M-CSF-dependent motility in in vitro scratch assays and trans-well migration assays.

To examine the myeloid-specific transcriptional regulation of Stap1, promoter assays were performed with different myeloid cell lines. Nested deletion assays and site-directed mutagenesis demonstrated an essential role of evolutionarily conserved PU.1 sites. In vivo binding of PU.1 to the Stap1 promoter region was demonstrated using chromatin immunoprecipitation. Furthermore, re-expression, and activation of PU.1 in PU.1(-/-) progenitor cells restored Stap1 transcription, indicating a direct role of PU.1 in the myeloid-specific regulation of Stap1.

Taken together, our results indicate that (i) activated microglia and macrophages express high levels of Stap1, (ii) Stap1 interacts with the M-CSF-receptor to enhance cellular activation, and (iii) the expression of Stap1 is critically dependent on the transcription factor PU.1.

P.025

E-CADHERIN AS A NOVEL MARKER FOR ALTERNATIVELY ACTIVATED (M2A) MACROPHAGES

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The polyvalence of macrophages is at least partly dependent on their ability to adopt diverse activation states in response to different stimuli, leading to the formation of pro-inflammatory classically activated (M1) macrophages in a Type I cytokine environment (IFN-g, TNF,…) and anti-inflammatory alternatively activated (M2) macrophages in the presence of Type II cytokines (IL-4, IL-13, IL-10,…). Previous studies from our lab established a consensus gene signature for in vivo-induced M2, including E-cadherin (Cdh1) as a new M2 marker.
E-cadherin has not been described in macrophages before, opening a window of opportunity to investigate E-cadherin regulation, protein expression/localization and function in these myeloid cells. IL-4 and IL-13, alone or in synergy with IL-10 and especially TGF-b, rapidly induce E-cadherin mRNA in murine macrophages. This Cdh1 induction is strictly dependent on the presence of the IL-4Ra chain and associated JAK/STAT6 signaling and is inhibited when de novo protein synthesis is blocked, suggesting the involvement of IL-4-regulated factors in E-cadherin gene induction. Indeed, inhibiting arginase1 and ornithine decarboxylase function results in strongly reduced Cdh1 induction by IL-4 and this defect is reversed by polyamine supplementation. Importantly, similar findings are observed in human macrophages. Regulation of E-cadherin mRNA directly correlates with the presence of E-cadherin protein at the cell surface as confirmed by flow cytometry and fluorescence microscopy. Although the mRNA levels of the E-cadherin-binding catenins are not influenced by M2-polarizing effectors, a functional E-cadherin/b-catenin/p120catenin complex appears to be formed at the cell surface. Evidence for the in vivo regulation of the E-cadherin/catenin complex in macrophages comes from findings that Taenia crassiceps metacestodes induce the expression of E-cadherin and its catenins in peritoneal macrophages from WT but not IL-4, IL-4Ra and STAT6 deficient mice. Interestingly, the altered macrophage E-cadherin/catenin expression during T. crassiceps infection resulted in an enhanced heterotypic adhesion of these M2 to CD103 or KLRG1-positive cell lines in vitro. To investigate the role of E-cadherin in macrophages in more detail, we generated E-cadherin over-expressing Raw264.7 clones, indeed indicating that enhanced E-cadherin expression enables these macrophages to interact with CD103 or KLRG1 positive cells. To evaluate the importance of macrophage E-cadherin in vivo, we generated macrophage-specific E-cadherin KO mice (LysMcre/cre x Cdh1lox/lox). Using these tools we are investigating the effect of macrophage E-cadherin expression on different intracellular signaling events and on the interaction of M2 with other cell types (epithelial cells, Treg, NK cells). Overall, our data provide a first insight in the regulation and function of E-cadherin/catenin expression in in vitro and in vivo-elicited macrophages.

P.026

IMMUNE CONTROL OF MUSCLE DAMAGE AND REPAIR: FROM MACROPHAGES POLARIZATION TO MESOANGIOBLASTS TRANSDIFFERENTIATION

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Mesoangioblasts are stem cells derived from vessels able to regenerate skeletal muscle. After injection in the blood circulation, mesoangioblasts migrate outside the vessels homing to damaged muscles, where they interact with macrophages. The role of macrophages that invade muscle after injury is poorly understood, although they consistently infiltrate both acute and chronic muscle damage. In models of acute injury macrophages have been clearly demonstrated to sustain muscle remodelling and regeneration: they dispose of necrotic fibres and deliver survival signals to myogenic precursors. However macrophages can also amplify muscle damage and macrophage depletion ameliorate the phenotype of mdx -/- mice (the murine model of Duchenne muscular dystrophy). Furthermore it is not known if macrophage and stem cells interact at the site of damage and whether their interaction play a role in the success (and potentially the failure) of muscle repair. In this work, taking advantage of co-culture and transwell systems, we characterized the reciprocal influence of the two cell populations. The presence of mesoangioblasts influences macrophages: they over-express the CD11b integrin, the CD163 haemoglobin scavenger receptor and RAGE (the receptor for advanced glycated endproducts and for the damage-associated molecular pattern, HMGB1). CD163 overexpression in macrophages is apparently linked to the autocrine/paracrine secretion of IL-10 and associates with macrophage ferritin overexpression (a characteristic that may allow macrophages to safely deal with the high amount of free iron present in the damaged muscle). Mesoangioblast survival in the damaged muscle and trans-differentiation in myotubes, necessary for efficient muscle repair were both facilitated by the interaction with macrophages: mesoangioblasts survival is increased by tropic factor produced by macrophages and they efficiently trans-differentiate in myotubes when co-cultured in the presence of macrophages that underwent functional polarization towards an M2 phenotype, associated with immunoregulation and tissue remodelling. On the contrary inflammatory (γ−INF activated) macrophages interfere with mesoangioblasts trans differentiation. All together, these results reveal a previously unknown circuit, by which stem cells modulate the macrophage plasticity while macrophages strongly influence mesoangioblast ability to survive, to differentiate and therefore to regenerate skeletal muscle fibers.

P.027

MACROPHAGE POLARIZATION BY S1P FROM APOPTOTIC TUMOR CELLS

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The presence of tumor-associated macrophages (TAM) often correlates with a poor survival prognosis for patients with solid tumors. TAM acquire an anti-inflammatory phenotype in response to the tumor microenvironment. We have evidence that apoptotic cell-derived sphingosine-1-phosphate (S1P) could be one of the factors, which contribute to TAM polarization.

During apoptosis, sphingosine kinase 2 (SphK2) is released from apoptotic cells after N-terminal cleavage by caspase-1. The release demands binding of SphK2 to phosphatidylserine (PS) at the inner leaflet of the plasma membrane and subsequent exposure of PS. Extracellular SphK2 then generates S1P in the interstitium. In a co-culture of human macrophages with MCF-7 breast carcinoma cells, apoptosis occurred in the latter ones, upon which they released S1P. Macrophages from these co-cultures acquired an alternatively activated phenotype, characterized by reduced TNF-α release as well as attenuated NF-κB DNA binding, but elevated IL-10 and IL-8 production. Apoptosis-resistant tumor cells neither released S1P, nor induced macrophage phenotype alterations. In analogy, when SphK2 was knocked down in MCF-7 cells, phenotype alterations were absent. Therefore, we propose that tumor cell death-derived S1P contributes to TAM polarization.

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P.028

IL-18 FUNCTIONAL AVAILABILITY DURING THE REGULATION OF MONOCYTE ACTIVATION

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Monocytes participate to innate immune responses and inflammatory reactions by initiating different activation programmes. Classically activated monocytes (M1) produce inflammatory cytokines and factors in response to bacterial components (e.g. bacterial LPS) and to inflammation-related cytokines (e.g. IFNγ). Upon resolution of the defence reaction, alternative activation (M2) by stimuli such as the anti-inflammatory cytokine IL-4 limits inflammation and initiates tissue reconstruction.

The regulation of monocyte activation has been examined by evaluating mRNA expression of the inflammatory Th1-polarising cytokine IL-18 and related genes upon exposure to inflammatory vs. anti-inflammatory stimuli (LPS, IFNγ, LPS + IFNγ, IL-4, IL-18) for 4 or 24 h.

LPS regulated IL-18 expression with a characteristic time-course, while IL-18BP expression was unchanged. IFNγ strongly induced expression of IL-18BP, while ineffective on IL-18 expression. The presence of IFNγ abolished the LPS-induced upregulation of IL-18 expression. Thus, LPS could induce an early increase of the IL-18/IL-18BP ratio (predictive of inflammation) and a decrease at later times (anti-inflammation). In the presence of IFNγ this ratio was rapidly and potently shifted towards anti-inflammation. LPS upregulated expression of other inflammatory genes (IL-1β, caspase-1), while IFNγ had no effect. On the anti-inflammatory gene IL-1Ra, IFNγ could delay LPS-induced stimulation. Thus, relative expression of inflammatory vs. anti-inflammatory genes varies depending on the microenvironment and can determine the outcome of the response.

Anti-inflammatory IL-1F7 binds non-competitively to the IL-18 receptor IL-18Rα, but cannot recruit IL-18Rβ. Of the five IL-1F7 splice variants, only isoforms a, b and d are putatively active. Fresh monocytes only express low levels of IL-1F7a, b, and c. Exposure to LPS or LPS+IFNγ increases expression of all three isoforms but with different time courses. IL-18 increases IL-1F7b and c expression at late times, but never increases IL-1F7a expression. On the other hand, IL-4 tends to increase IL-1F7a and b expression at late times. Expression of TIR8 (an inhibitory receptor of the IL-1R family) is down-regulated by LPS and LPS + IFNγ, while IL-4 tends to increase it.

It is hypothesized that TIR8 may be a co-receptor for IL-1F7. Their possible interaction is being studied in silico by molecular modelling. The complex of IL-1F7 with IL-18Rα has been docked using the homology models of IL-1F7b (built on the IL-18 NMR structure) and of the receptor (built on the IL-1RI crystal structure). The model of TIR8 has been built on the third domain of IL-1RI. Docking of TIR8 to the IL-1F7/IL-18Rα complex, using AutoDock4.0 and GROMACS 3.3.1, is currently in progress.

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P.029

DIFFERENTIAL BETA-ARRESTIN 2 (ARRB2) EXPRESSION DURING REJECTION OF EXPERIMENTAL RENAL ALLOGRAFTS

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Numerous leukocytes accumulate in the blood vessels of experimental renal allografts. A majority of them are identified as activated, cytotoxic monocytes, which may cause graft rejection. To elucidate the mechanisms of monocyte activation during allograft rejection, we sought to identify novel regulating factors. Renal transplantation was performed in Lewis rats, and Dark Agouti rats were used as allogenic donors. Intravascular graft leukocytes were obtained from isografts and allografts by perfusion on day 4 after transplantation. RNA extracted from perfusate leukocytes was used for microarray analyses of genes regulated during acute rejection. Microarray technology and quantitative RT-PCR revealed that mRNA expression of ARRB2 was reduced in leukocytes from allografts compared to isografts. Differential expression of ARRB2 protein was confirmed by Western blotting. In agreement with these findings, also the mRNA level of beta 2 adrenergic receptor which regulates the level of ARRB2 was markedly decreased in these cells. Confocal immunofluorescence microscopy revealed ARRB2 expression in graft monocytes and other leukocytes and the regulation of ARRB2 by monocytes was further confirmed by two-colour FACS analysis. Since ARRB2 can serve as an endogenous inhibitor that blocks signal-induced IkappaB degradation and subsequent activation of NFkappaB, we examined the expression of genes controlled by this transcription factor. We observed an up-regulation of TNF alpha, IL-1 beta and iNOS mRNA in cells from allografts. Concomitantly, IkappaB levels were significantly reduced during rejection. Our results suggest that the NFkappaB mediated activation of monocytes in the blood vessels of renal isografts is blocked by the presence of high levels of ARRB2. During acute rejection, however, ARRB2 levels are drastically reduced and classical monocyte activation via NFkappaB is enabled.

P.030

DECREASED EXPRESSION OF HLA-DQ ON BLOOD MONOCYTES AND SPUTUM MACROPHAGES IN PATIENTS WITH CYSTIC FIBROSIS VERSUS HEALTHY SUBJECTS

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Background: The major histocompatibility complex DQ (HLA-DQ) is found on antigen presenting cells such as dendritic cells, monocytes and macrophages. It interacts with T-cell receptors (TCR) to present foreign as well as self antigens to T-helper cells. Since proper expression of HLA class II molecules is required for an efficient defence against infection we studied expression of these molecules in patients with cystic fibrosis (CF) because these patients suffer from repeated airway infection. Study design and methods: We examined the expression of HLA-DQ, and -DR in patients with CF (homozygous deltaF508) versus healthy subjects at the protein level (flow cytometry) and mRNA level (RT-PCR) on blood monocytes as well as on sputum macrophages. Results: In 20 healthy donors we found an HLA-DQ expression with an average fluorescence intensity of 4.25 ± 2.23 channels compared to 20 deltaF508 homozygous CF patients with 2.01 ± 1.13 channels ($p< 0.0022$). Within these 20 CF patients we found a subgroup of 13 which showed a very low HLA-DQ expression lower than 2 channels (average 1.41 ± 0.64) and a subgroup of 7 cases with near normal expression (3.12 ± 1.01). In CF patients who express low levels of HLA-DQ on blood monocytes, expression of DQ on sputum macrophages was low as well (0.44 ± 0.66), and in CF patients who express higher levels of HLA-DQ on blood monocytes also showed higher HLA-DQ expression levels on sputum macrophages (2.07 ± 1.16). HLA-DR expression showed a high variability but its expression was lower in CF patients (18.37 ± 6.99) compared to healthy subjects (27.08 ± 15.65, $p=0.024$). At the mRNA level no differential expression of HLA-DQalpha1, -DQbeta1 or DRalpha was detected between the two groups. This implies that the pronounced down regulation of HLA-DQ in the majority of patients occurs post-transcriptionally. The data suggest that the reduced expression of Class II MHC molecules may contribute to the increased rate of infections seen in CF.

P.031

LACTIC ACID INHIBITS TNF SECRETION BY HUMAN MONOCYTES VIA SUPPRESSION OF GLUCOSE METABOLISM

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High lactate concentrations are found under various pathophysiological conditions. Here, we analyzed the influence of lactic acid on the secretion of TNF by human monocytes. Cells were cultured with LPS in the absence or presence of lactic acid, which inhibited the secretion of TNF in a dose dependent fashion. These results were confirmed in a coculture model of monocytes with multicellular tumor spheroids. Blocking tumor-derived lactate by oxamic acid, an inhibitor of lactate dehydrogenase, reverted the suppression of TNF secretion. Next, we investigated possible mechanisms underlying this suppression. Uptake of [3-13C]lactate by monocytes was shown by hyphenated mass-spectrometry. As lactate might interfere with glycolysis, the glycolytic flux of monocytes was determined. We added labelled [1,2-13C2]glucose to the culture medium and measured glucose uptake and conversion into [2,3-13C2]lactate. Activation of monocytes increased the glycolytic flux and the secretion of [2,3-13C2]lactate. Addition of unlabelled lactic acid resulted in a significant decrease in [2,3-13C2]lactate secretion, while intracellular [2,3-13C2]lactate levels increased, suggesting a disturbed lactate export. Accordingly, the blockade of lactate transport by alpha-cyano-4-hydroxycinnamic acid or inhibition of glycolysis by 2-deoxyglucose also inhibited TNF secretion. Furthermore, lactic acid strongly diminished the intracellular ATP level of activated monocytes. Our data suggest that high levels of extracellular lactic acid lead to an inhibition of lactate export. As a consequence, glycolysis and ATP production in monocytes are inhibited, which in turn suppresses cellular activation. These results support the hypothesis that high concentrations of lactic acid in an inflammatory environment suppress monocyte activation via inhibition of glycolysis.

P.032

SUCCESSFUL PREVENTION OF SURGERY-INDUCED LIVER METASTASES DEVELOPMENT AFTER ANTI-TUMOR MONOCLONAL ANTIBODY THERAPY IS MEDIATED BY THE INNATE MONONUCLEAR PHAGOCYTE NETWORK

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Introduction - Liver metastases are a frequent complication of colorectal cancer (CRC), often even after successful resection of the primary tumor at a time at which no visible metastases are present. Post-operative adjuvant treatment, aimed to eliminate residual disseminated tumor cells, may help prevent secondary disease. As antibody therapy has been acknowledged as a successful strategy to treat malignancies, we studied the potential of therapeutic antibodies to prevent outgrowth of liver metastases in immuno-competent mice and rats

Methods - Liver metastases were induced in mice via injection of murine B16F10 melanoma cells into the spleen or by injection of rat CC531s colon carcinoma cells into RI/III-/- orγRIII-/-, FcγRI-/-, Fcγthe mesenterial vene in rats. Wild type, Fc RI/II/III-/- mice were treated with anti-gp75 antibody (TA99). Alternatively,γFc rats were treated with a low (3 x 10 µg) or high (3 x 100 µg) dose of anti-CC531s antibodies of different isotypes (MG4-γ1 (mIgG1), MG4-γ2a (mIgG2a) and MG4-γ2b (mIgG2b)). Liver macrophages (Kupffer cells, KC) were depleted in mice and rats by means of i.v. clodronate liposome injection. The number of liver metastases was determined 3 weeks after tumor cell inoculation. To study short term events after surgery, animals were sacrificed 24 hours after injection of fluorescently-labeled tumor cells.

Results - We showed that anti-tumor antibodies efficiently prevent liver metastases outgrowth in both mice and rats. Additionally, efficacy of antibody therapy was dependent on the presence of the IgG receptors FcγRI and FcγIV. Because these receptors are exclusively expressed by cells of the innate mononuclear phagocyte network, we further investigated the role of both monocytes and macrophages in antibody therapy of liver metastases. Less tumor cells were present in livers of control rats that had been treated with antibody 24 hours after tumor cells injection. However, after KC depletion no difference was observed in tumor cell numbers in antibody-treated or non-treated rats, supporting a prominent role for KC. Moreover, treatment with a low antibody dose (3 x 10 µg) was sufficient to prevent liver metastases outgrowth in control rats, but therapeutic efficacy was completely abrogated in KC depleted animals. Interestingly, when high doses (3 x 100 µg) were injected in KC depleted rats, antibody treatment still partly prevented metastases outgrowth, which was due to phagocytosis of tumor cells by recruited monocytes.

Conclusion - Antibody treatment after surgery can efficiently prevent the development of liver metastases. The protective effect of antibodies is mainly mediated by KC, although monocytes are able to partly replace KC when high doses of antibody are administered. The discovery that KC and monocytes can eliminate tumor cells after surgery through antibody-dependent cellular cytotoxicity has promising clinical implications for designing new adjuvant therapies for patients with CRC.

P.033

GLUCOCORTICOIDS PROMOTE SURVIVAL OF ANTI-INFLAMMATORY MONOCYTES VIA AUTOCRINE STIMULATION OF ADENOSINE A3 RECEPTOR

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Glucocorticoids (GC) are widely used anti-inflammatory and immunosuppressive agents, acting on many cells of the immune system, including monocytes and macrophages. However, the exact mechanism of GC action on monocytes is still not completely elucidated. Analysis of the general GC-induced expression pattern in human monocytes by microarray technology revealed that the main GC effect in monocytes is not suppression of pro-inflammatory mediators. GC rather induce a hitherto undescribed active anti-inflammatory phenotype, which seems to play a pivotal role in resolution of inflammation. Consistently, GC-treatment did not limit monocyte viability, but on the contrary, promoted their survival and protected them from apoptosis induced by various stimuli. Investigation of this novel anti-apoptotic effect of GC on monocytes showed that GC-treatment led to delayed and sustained phosphorylation of ERK/MAPK pathway without affecting other intracellular kinase cascades. Importantly, GC-mediated activation of ERK/MAPK pathway correlated with increased resistance to apoptosis and ERK1/2 inhibition restored monocyte susceptibility to apoptosis. We identified A3 adenosine receptor (A3AR) as the initial component of this anti-apoptotic signal transduction pathway. GC up-regulated expression of A3AR and triggering of A3AR was responsible for activation ERK1/2 phosphorylation. Importantly, specific inhibition of signalling from A3AR completely abolished GC-induced ERK1/2 activation and resistance to apoptosis. Thus, our results demonstrate that adenosine-induced survival of anti-inflammatory monocytes is a novel mechanism by which GC are capable of down-regulating inflammatory processes.

P.034

PHYSIOLOGICAL AND PATHOLOGICAL CONSEQUENCES OF IMMUNOGLOBULIN A- INDUCED MIGRATION

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Immunoglobulin A (IgA) is the principal antibody present in mucosal areas and plays a key role in mucosal defense. The Fc receptor for IgA (FcalphaRI) is expressed by myeloid cells, including neutrophils and monocytes, and can trigger both pro- and anti-inflammatory responses in vitro. Despite considerable progress in understanding the role of FcalphaRI on monocytes, functions of neutrophil FcalphaRI are ill-understood. We now demonstrate that IgA has a previously unrecognized role in mediating migration, since cross-linking of neutrophil FcalphaRI by IgA leads to release of leukotriene B4 (LTB4), which is one of the most potent chemoattractants for neutrophils. Moreover, after cross-linking of FcalphaRI, neutrophils secrete monocyte chemotactic stimuli, resulting in enhanced migration of monocytes. Dimeric IgA (dIgA), but neither secretory IgA nor IgG, was equally capable of inducing neutrophil recruitment. As such, dIgA functions as an inflammatory antibody at the baso-lateral membrane of epithelial cells in the mucosa, whereas SIgA serves as a non-inflammatory antibody at the luminal surface. Because neutrophils are the first cells that arrive at inflammatory sites once pathogens have crossed the epithelial barrier, we propose that dIgA-FcalphaRI interactions on neutrophils constitute an essential activation step to recruit more neutrophils as well as monocytes in order to help clearing the impending infection.

However, in several diseases aberrant IgA depositions are observed in tissues. For instance, the chronic autoimmune blistering disease 'linear IgA bullous disease' is characterized by IgA directed against the epidermal basement membrane in the skin. Furthermore, large (FcalphaRI-expressing) neutrophil infiltrates are present at the border between the epidermis and dermis, with concomitant blister formation. We now show that the presence of IgA complexes in either ex vivo human skin or in vivo results in neutrophil accumulation, which is dependent on interaction with FcalphaRI. Because recruited neutrophils will also encounter IgA deposits in the skin, and respond with secretion of even more LTB4, a perpetuating inflammatory loop will be sustained, which will seriously aggravate morbidity.

In conclusion, under physiological circumstances dIgA-induced neutrophil migration may result in elimination of invading micro-organisms, hereby helping to maintain gut homeostasis. However, in diseases with aberrant IgA deposits (like the skin), IgA-induced neutrophil migration can contribute to severe tissue damage.

P.035

M2 TYPE MACROPHAGES, POLARISED WITH M-CSF AND IL-10, PHAGOCYTOSE RITUXIMAB OPSONISED LEUKAEMIC TARGETS MORE EFFICIENTLY THAN M1 CELLS

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Since macrophages have been implicated as major players in the mechanism of action of Rituximab (Mabthera®) in vivo, we have investigated the factors that modulate their tumour cell killing potential in vitro. Human macrophages expressing CD16, CD32 and CD64, were differentiated from CD14+ peripheral blood monocytes by culture for 5-7 days in presence of M-CSF. Binding of rituximab opsonised target cells was measured after 5 minutes incubation of macrophages with CFSE labelled B-CLL cells and FACS analysis. Phagocytosis was quantified after 2 hours at 37°C by microscopic count of stained slide preparations. Rituximab induced specific binding of CD20+ target cells to macrophages and this was followed by phagocytosis, but not ADCC. Phagocytosis was maximal at 0.1 µg/ml rituximab and was not significantly affected by CD20 expression levels on target cells. The CD16A polymorphism at amino acid 158 (Val/Phe) that affects IgG binding did not significantly modify the efficacy of phagocytosis by M2 cells at different rituximab doses, possibly due to the role of additional Fcγ receptors. Indeed phagocytosis was blocked by excess human immunoglobulins. Since macrophages can be differentiated to M1 type or M2 type cells with either GM-CSF or M-CSF, respectively, and can be classically activated by pro-inflammatory stimuli (IFNγ + LPS) or undergo alternative activation with cytokines such as IL-4 or IL-10, we have analysed the effect of these different polarisation programs on the phagocytosis mediated by rituximab. Macrophages differentiated in presence of M-CSF showed a 2-3 fold greater phagocytic capacity compared to GM-CSF induced cells. Furthermore addition of IL-10 significantly increased, whereas IL-4 decreased phagocytic capacity of both M-CSF and GM-CSF differentiated macrophages. LPS/IFNγ had little effect. Expression of CD16, CD32A/C and CD64 correlated with the phagocytic capacity of the different polarised populations, suggesting that M-CSF and IL-10 induced maximal phagocytosis through upregulation of several activating FcγRs. Since tumour infiltrating macrophages are usually of the M2 type and favour tumour growth, the data presented suggest that rituximab may convert these macrophages from tumour promoting to tumour suppressing cells. The possible clinical implications of these findings will be discussed.

P.036

FUNCTIONAL PROFILE OF TLR IN TUMOR ASSOCIATED MACROPHAGES

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Macrophages are crucially involved in immunosurveillance against pathogens and malignancies. They recognize the invading pathogens and initiate the immune-inflammatory reaction through pattern recognition receptors (PRR). Toll-like Receptors (TLR) represents one of the most important PRR family that recognizes a diverse range of molecules derived from pathogens and host cells (Fig. 1). As the number and diversity of TLR ligands and host factors increases, more questions are being raised. The role of TLR in cancer is dispersed. Clinical evidence have shown increased susceptibility of tumour patients to infection, but is still unknown if this phenomenon correlates with alteration in the TLR repertoire or is a general effect of the immunosuppressive state induced by tumour. On the therapeutic front, TLR ligands are being evaluated as potential adjuvants in cancer therapies. TAM (Tumor-Associated Macrophages), constitute the major portion of the lymphoreticular infiltrates of most solid tumors and play a central role in the host immunity-tumor interaction. Supporting the protumoral nature of TAM, our group has described that TAM display defective expression of inflammatory cytokines in response to lipopolysaccaride (LPS), in both murine (fibrosarcoma) and human (ovarian carcinoma) tumors. Based on this finding, we decided to investigate the TLR repertoire of TAM from ovarian carcinoma patients, as compared to monocytes and monocytes-derived macrophages. As result, we observed that TAM ascites express significant level of TLR2 mRNA, while only poor or absent expression of other TLR members (TLR1-TLR9) was detected. TLRs are potential target of immunotherapy against cancer and ligands for TLR9 (CpG) or TLR4 (Taxol) are currently considered in clinic as anticancer drugs. Based on this we investigated the expression of TLR4, TLR9 and TLR2 in PBMCs from both healthy donors and tumor patients, as well as in TAM from both primary ovarian carcinoma and omentum metastasis. Only TLR2 was confirmed to be expressed at significant level in these specimens. Finally, ex vivo experiments demonstrated that activation of TAM with the TLR2 agonist PAM3CSK4 induced higher level of proinflammatory cytokines (e.g.: TNFa), as compared to TLR4 (LPS from S. abortuus equi) and TLR9 (human: CpG2006; murine: CpG1826) agonists. Our studies suggest that in neoplasia TLR2 ligands could have a major proinflammatory activity and could induce a better immunotherapeutic response compared to TLR9 ligands.

P.037

DIVERGENT EFFECTS OF HYPOXIA ON DENDRITIC CELL FUNCTIONS

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Dendritic cells (DC) are professional antigen-presenting cells (APC) patrolling tissues to sense danger signals and to activate specific immune responses. In addition they play a role in inflammation and tissue repair. Here we show that oxygen availability is necessary to promote full monocyte-derived DC differentiation and maturation. Low oxygen tension (hypoxia) inhibits expression of several differentiation and maturation markers (CD1a, CD40, CD80, CD83, CD86 and MHC class II molecules) in response to lipopolysaccharide (LPS), as well as their stimulatory capacity of T cell functions. These events were paralleled by impaired upregulation of the chemokine receptor CCR7, a necessary event for the homing of mature DC to lymph nodes. In contrast, hypoxia strongly inβ and IL-1αup-regulates production of pro-inflammatory cytokines, TNF particular, and the inflammatory chemokine receptor CCR5. Subcutaneous injection of hypoxic DC in the footpad of mice resulted in enhanced leukocyte recruitment at the site of injection and defective DC homing to draining lymph nodes. Thus hypoxia uncouples the promotion of inflammatory and tissue repair from sentinel functions in DC, a safeguard mechanism against self-reactivity of damaged tissues.

P.038

ROLE OF P50 NF-KB IN DENDRITIC CELL FUNCTIONS

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Tumor associated dendritic cells (TADC) and tumor associated macrophages (TAM), are important immune components of the tumour stroma. Under normal conditions, dendritic cells (DC) in particular, but also macrophages, play a crucial role in triggering inflammation and promoting adaptive immune response, through the presentation of antigens to T cells. Nevertheless, DC present in tumors have been often associated with tolerogenic functions. In this regard, while DC maturation was reported to require NF-kB activation, several line of evidence demonstrated that this pathway is strongly impaired in infiltrating leukocytes, including TADC and BκTAM. In the attempt to elucidate the mechanism driving defective NF-activity in tumour infiltrating leukocytes, we have recently reported that TAM display massive nuclear localization of the p50 NF-Kb inhibitory homodimer, which correlates with impaired inflammatory functions. The functional significance of this observation was demonstrated in p50 NF-kB deficient mice, which displayed tumor growth inhibition. More recently, to evaluate whether this tolerogenic mechanism may target other compartments of the immune system, we characterized the role of p50 NF-kB in dendritic cells functions, including their differentiation and maturation. Our data indicate that the p50 NF-kB homodimer has inhibitory functions in bone marrow derived DC, since its depletion restores pronounced inflammatory and co-stimulatory properties, both in vitro and in vivo. Preliminary results also suggest that inhibition of p50 NF-kB homodimer formation in TADC significantly abrogates their tolerogenic properties. Our study suggest that targeting microenvironmental signals and pathways leading to upregulation of p50 NF-kB homodimer in TAM and TADC may represent a novel strategy to restore antitumour functions in vivo.

P.039

ARGINASE I INDUCTION BY MODIFIED LIPOPROTEINS IS MEDIATED BY PPAR ACTIVATION IN MOUSE AND HUMAN CELLS.

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The uptake of modified lipoproteins by macrophages is an important early event in the development of atherosclerosis. We analyzed the ability of modified LDL (oxidized and acetylated) to alter the expression and activity of Arginases (ArgI and ArgII) in macrophages. We show that ArgI expression is potently induced by both oxLDL and acLDL and that this effect is mediated by peroxisome proliferator-activated receptors (PPARs). Arginase I expression is highly responsive to agonists for PPARgamma and PPARdelta but not PPARalpha. Moreover, the induction of ArgI by both PPAR agonists and IL-4 is blocked in macrophages from PPARgamma- and delta-deficient mice. The dependence of Arginase I expression by PPARgamma/delta in foam cells reinforces previous studies in which have been demonstrated the contribution of these nuclear receptors to switch macrophage phenotype and reprogram their activation status. The same is the case for macrophage arginase I induction, which have been proved to be a major component of this alternative status, triggered in the context of a predominant Th2 response. Finally we have demonstrated that Arginase I could be also induced by oxLDL and PPAR ligands in human macrophages in absence of Th2 cytokines, reinforcing the hypothesis that these nuclear receptors are important transcriptional regulators of macrophage differentiation.

P.040

ANTI-TUMOR AND ANTI-INFLAMMATORY EFFECTS OF TRABECTEDIN ON HUMAN LIPOSARCOMA CELLS

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We reported that the marine-derived anti-tumor agent Trabectedin (ET-743, Yondelis) has selective anti-inflammatory properties which can be of advantage in view of the association between chronic inflammation and tumor progression. Trabectedin is currently used in Phase II/III studies in patients with ovarian , breast cancer and sarcoma. In this study we investigated the peculiar susceptibility of human liposarcoma cells to Trabectedin. Two cell lines (402.91 and 1765) and 7 primary cultures derived from surgical specimens of liposarcoma were highly susceptible to the cytotoxic effect of Trabectedin at nM concentration. Both drug treated liposarcoma cell lines were studied by a TaqMan® low density array with 92 apoptosis-related genes to gain more insight on the mechanisms of drug-induced death. Several pro-apoptotic genes (e.g. FAS,CASP10,TNF,LRDD) were up-regulated whereas the anti-apoptotic gene BCL2 was down-regulated by Trabectedin at 18hours but not at earlier time points. Transcriptional activity of selected cytokine/chemokine genes is affected by this drug. Moreover, Trabectedin significantly reduces CCL2 and the inflammatory protein Pentraxin3 (PTX3) either at transcriptional and protein level, especially after TNFa/IL1B stimulation. Down-regulation of PTX3 was confirmed in primary culture of liposarcoma and its serum levels are measured in patients undergoing clinical treatment with drug. We further studied the activity of Trabectedin on the cross-talk between tumor cells and monocytes/macrophages. The enhanced survival and proliferation of liposarcoma cells co-cultered with monocytes were inhibited by pretreatment with Trabectedin. Overall these results indicate an additional interesting value of this anti-tumoral drug: besides its direct cytotoxic effect on tumor cells, Trabectedin also affects the transcriptional activity of inflammatory genes and the interplay between tumor and stromal cells.

P.041

CAPNOCYTOPHAGA CANIMORSUS IMPAIRS THE ABILITY OF MACROPHAGES TO CLEAR BACTERIAL INFECTIONS

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Capnocytophaga canimorsus is a gram-negative commensal living in the oral cavity of dogs and cats. If transmitted to humans by bites, scratches, or indirect contacts, it can cause fatal septicemia or meningitis. Previous work done in our lab has shown that C. canimorsus 5 (Cc5), a strain isolated from a patient with fatal septicemia, survives and feeds on human and mouse macrophages without inducing a proinflammatory response or cellular cytotoxicity (Shin et al., 2007; Mally et al., submitted).

We have previously shown that Cc5 is resistant to phagocytosis and killing by J774.1 mouse macrophages in a dose-dependent manner (Meyer et al., in press). In this work, we investigated whether Cc5 can prevent phagocytosis and killing of other bacteria by interfering with the macrophage's signalling cascade. Indeed, pre-infection of J774.1 with Cc5 (moi 50) before subsequent infection with E. coli led to a blockage in E. coli killing, while phagocytosis of E. coli remained unaffected. Upon growth in the presence of J774.1, Cc5 releases soluble factor(s), which is heat-labile and proteinase K treatment sensitive. Moreover, the addition of EDTA abolished the effect of Cc5 on the killing of E. coli. Altogether, this suggests that Cc5 not only resists phagocytosis by professional phagocytes but also impairs the ability of macrophages to clear bacterial infections.

P.042

NOD LIKE PROTEINS ARE MODULATED IN BOVINE MACROPHAGES AND DENDRITIC CELLS FOLLOWING INFECTION WITH *SALMONELLA ENTERICA* SEROVAR TYPHIMURIUM

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Salmonella enterica serovar Typhimurium is the main *Salmonella* species that infects human and cattle. It is zoonotic and causes similar symptoms in humans and cattle, mainly gut pathology and diarrhoea. Macrophages (MΦ) and dendritic cells (DC) are antigen-presenting cells (APC) and both cell types clearly play an important role in the immune response to *Salmonella* in human and bovine hosts. MΦ and DC link innate and acquired immune responses by performing a variety of functions including phagocytosis, secretion of cytokines and antigen presentation. The aim of this project is to investigate how MΦ and DC interact in the initial stages of infection in cattle with *Salmonella*. It is planned to use a functional genomics approach by comparing samples from non-infected MΦ and DC with MΦ and DC infected with heat-inactivated and live bacteria using the bovine global Affymetrix microarray. In addition, we are investigating the role of Nod like receptors (NLRs) in the early stages following infection of MΦ and DC with *Salmonella*. Because of their recent discovery, NLRs are not fully represented on the Affymetrix microarray. NLRs are a group of pattern recognition receptors (PRRs) including NOD1, NOD2, NOD3 and IPAF which are known to be associated with the host response to intracellular invasion by bacteria. Preliminary studies show these NLRs are expressed in bovine APC, but they are expressed at different levels in monocytes, DC and mΦ. We have found NLR expression is modulated in response to stimulation with heat-killed bacteria and bacterial endotoxins. In addition, there are differences in NLR expression between cattle breeds that differ in disease resistance. Our studies will provide new insights into early host-pathogen interactions in salmonellosis in cattle.

P.043

IGA ACTIVATED PMNS INDUCE MONOCYTE RECRUITING AND ACTIVATION

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Introduction - Immunoglobulin A (IgA) is the main antibody class present in the intestinal tract and plays a key role in mucosal defence. It is commonly regarded as non-inflammatory antibody without activation properties. However, it is now becoming increasingly clear that IgA and its Fc receptor FcαRI (CD89) possess potent pro-inflammatory functions. Previous work from our group showed that crosslinking of FcαRI on polymorphonuclear cells (PMNs) with IgA leads to activation of PMNs and release of LTB4, which represents a major chemoattractant for PMNs. Here, we investigated the effects of IgA-activated PMNs on monocyte recruitment and activation of resident tissue macrophages.

Methods - Primary human PMNs and monocytes were isolated from healthy volunteers. PMNs were stimulated for 60 minutes with IgA-, IgG- or bovine serum albumin (BSA)-coated sepharose beads. Supernatant was collected and monocytes were incubated for 48 hours with PMN supernatants with or without addition of lipopolysaccharide (LPS) (10ng/mL). Subsequently, cytokine production by monocytes was determined with ELISA. Alternatively, monocyte migration towards PMN supernatants was tested using a blind well chemotaxis chamber.

Results - PMNs migrated towards IgA-, but not IgG- or BSA- coated beads. Additionally, higher migration of monocytes towards supernatants derived from PMNs stimulated with IgA-coated beads was found, compared to IgG-coated beads. Monocytes were activated as well, since elevated production of pro-inflammatory cytokines, including IL-6, IL-12 and TNFα, was observed.

Conclusion - PMNs that are activated by binding of IgA to their FcαRI are capable of inducing monocyte recruitment and activation. When micro-organisms have crossed the epithelial barrier of the intestinal tract, PMNs interact with IgA-opsonized pathogens, which leads to secretion of monocyte chemoattractants. Consequently, monocytes are recruited and activated to the site of inflammation in order to help clearing the impending infection.

P.044

TUMOR-DERIVED HIGH MOLECULAR WEIGHT M-CSF INDUCES MONOCYTE DIFFERENTIATION INTO M2-POLARIZED MACROPHAGES

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Experimental and clinical evidence has highlighted that tumor-associated macrophages (TAM) represent the principal component of the leukocyte infiltrate and are usually associated with tumour growth, progression and metastasis. Macrophage population is generally divided into two distinct subsets: M1 and M2. M1 macrophages act as a first line of defence against pathogens whereas M2 cells participate in wound repair and maintenance of tissue integrity.

In the tumour micro-environment TAM interactions with the extracellular matrix, neighboring cells, and soluble stimuli largely influence their gene expression and behavior.

To investigate the role of the tumor micro-environment on macrophage differentiation, we cultured freshly isolated human monocytes with pancreatic cancer cell line supernatants, in the absence of exogenous cytokine addition.. In selected cultures, about 50% of the monocytes differentiated after 5 days into macrophages. The phenotype analysis of tumor-conditioned macrophages (TC-macro) demonstrated high expression of the mannose receptor, CD16, CD68 and low levels of MHC class II. TC-macro produced IL-10, IL-6, TNF but not IL-12, even after LPS stimulation. Moreover, TC-macro produced a panel of chemokines including CCL2, CXCL8, CCL17 and CXCL10. The transcriptional profile of TC-macro revealed that several genes in line with an M2 polarization are highly expressed. The nature of the tumor-derived factors inducing macrophage differentiation is currently under investigation; biochemical analysis indicated that the biological activity is excluded from exosomes and have a high molecular weight (>100.000 KDa). IL-3 and IL-6 were not detectable in tumor supernatants whereas M-CSF was present at low levels. By mass spectrometric techniques, we surprisingly found that the tumor-derived M-CSF had peculiar migration patterns which were different from those expected for the common human homodimeric glycosilated protein, suggesting an interesting structural differences for the tumor-secreted isoforms of this primary regulator of mononuclear phagocyte. The characterization of tumor-derived factors inducing macrophage differentiation could better clarify the intricate cross-talk between tumor cells and macrophages and thus might aid in the process of devising novel anti-tumor treatments.

P.045

RELEVANCE OF SIALIC ACID IN DENDRITIC CELL ENDOCYTOSIS PROCESS AND MATURATION

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Dendritic Cells are central to the development of immune responses, since they have the capacity to recognize, engulf and process foreign materials, and subsequently maturate and move to lymph nodes where they present processed antigens, triggering a T-cell response. For these reasons DCs based cancer vaccines offer the potential for an effective, non-toxic, and outpatient approach to cancer therapy. Although the use of DC based immunotherapy is being delayed due to the complexity and deficient knowledge of their biology. Given an increasing evidence of the involvement of sialic acids, the typical ending sugars of glycoprotein glycans, in the immune response, we are particularly interested in clarifying its influence in DC's function. We have previously observed an increased expression of sialylated structures during the differentiation of human monocyte derived-DCs (mo-DCs), which suggests a role for sialylation in DC biology. Additionally, we have observed that the removal of the sialylated structures by neuraminidase treatment decreases the capacity of mo-DC to endocytose certain antigens, known to be mainly uptaken by macropinocytosis (1). Our latest results show that the neuraminidase treatment, by itself, enhanced the expression of maturation markers such as MHC class I and II and the costimulatory molecules, CD80 and CD86, and also triggered the transcription of specific cytokine genes. These data suggest that the decrease of sialic acid content on cell surface, by neuraminidase, creates an overall pro-inflammatory effect on mo-DCs, increasing their immunogenicity. Since we had indications that the sialyltransferases (ST) ST3GalI and ST6GalI are probably the most relevant STs for DCs sialylation (1) we also focused our study on bone marrow derived DCs from ST3GalI and ST6GalI deficient mice. In both cases, an increased expression of MHC II, was observed confirming the more mature phenotype of DCs lacking these type of sialylated structures. Similarly to human mo-DCs treated with neuraminidase, DCs from these ST deficient mice presents a moderate endocytosis capacity. It is possible that the increased maturation phenotype, observed in DCs with decreased sialylation, diminished the capacity for antigen uptake, explaining their modest endocytosis ability. However, we observed that some endocytosis processes, such as the phagocytosis of microorganisms, are not negatively affected by the removal of sialic acid. Thus, the lack of these structures has possibly an additional negative effect in particular uptake mechanisms of DCs and we are conducting more thoughtful experiments to clarify this assumption. Even though, the results obtained so far, reveal that DC immunogenicity can be modulated by altering the sialic acid content, in particular, ST3GalI- and ST6GalI-mediated structures, suggesting sialylation as a pertinent issue to be considered in the establishment of DC based cancer vaccines. (1) Videira et al, 2008, Glycoconj J 25:259-268

P.046

PROFILE OF INFLAMMATION-RELATED GENES IN THE MICROENVIRONMENT OF HUMAN COLON CANCER

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Experimental and epidemiological studies indicate that tumor-associated inflammation promotes disease progression in colon cancer, the second largest cause of cancer-related death in Western countries. It has been extensively demonstrated that several growth factors and cytokines play pivotal roles in the regulation of proliferation, survival, adhesion and migration of neoplastic cells. On the other hand, the presence of infiltrating leukocytes (CD8 memory T-cells) in colon cancers has been associated with better prognosis. To investigate which inflammatory mediators are mostly expressed in the tumor microenvironment, we have analyzed the RNA profile from colon tumor samples and their respective normal tissues, using TaqMan Low Density Array (Applied Biosystem), customized with 91 inflammation related genes. In a preliminary series of eight tumor samples, of which four with micro-satellite instability (MSI), the most frequently up-regulated genes were chemokines: CXCL10, CXCL8 and its receptor CXCR2; cytokines: IL-24, IL-23, IL-1, IL-1Ra; and matrix-associated proteins: SPARC and osteopontin (OPN). Up-regulated expression of CXCL8, IL-23A, OPN, SPARC and IL-24 mRNA was subsequently confirmed in other thirty colon cancer samples analyzed by quantitative real-time PCR. Interestingly, several preliminary data indicate that the majority of up-regulated genes are produced by infiltrating cells, both lymphocytes and macrophages, suggesting their pivotal role in colon cancer progression. The investigation of these inflammatory mediators expressed in the microenvironment could lead to the identification of novel prognostic markers and potential targets for innovative cancer therapies.

P.047

KINETIC AND CYTOKINE PROFILE OF CD4+FOXP3+ REGULATORY T CELLS IN PERIPHERAL LYMPHOID ORGANS AND CNS DURING THE COURSE OF RELAPSING EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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CD4+Foxp3+ T regulatory cells are believed to play a relevant role in determining occurrence and disease course in autoimmune processes such as Multiple Sclerosis (MS) and its animal model experimental autoimmune encephalomyelitis (EAE). During EAE, myelin-reactive pro-inflammatory CD4+ T cells infiltrate the CNS and, in concert with other mononuclear cells, cause inflammation and progressive paralysis. Although several studies clearly demonstrate the ability of Foxp3+ T cells to prevent EAE in mice, not many address their phenotypic characteristics and their mechanism of action. We have performed a kinetic and cytokine analysis of the cellular composition of secondary lymphoid organs and of the central nervous system (CNS) at different time points after relapsing-remitting EAE induction in SJL by immunization with PLP139-151. We describe the kinetic and possible migration features of different subpopulation of CD4+Foxp3+ T cells during the course of the disease. We also, surprisingly, found that, approximately 10-12% of Foxp3+ T cells were positive . Cytokine . intracellular cytokine staining for IL-2, IL-17, or IFNγ by positive CD4+Foxp3+ T cells were enriched in the CD25+, CD25-, CD103+ subpopulations. Our results, showing a small but consistent percentage of T cells double positive for Foxp3 and effector cytokines, questions the concept of Foxp3 as a marker tout court associated to suppressive functions also in mice.

P.048

RATIONAL DESIGN OF SYNTHETIC PEPTIDES FOR GENERATION OF ANTIBODIES THAT RECOGNIZE CD11c+ HORSE DENTRITIC CELLS IN LYMPH NODES

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Integrins are a family of adhesion molecules composed by eighteen α subunits and eight β subunits which in mammals can ensamble together to form 24 known distinct heterodimers. They are involved in a great diversity of immunological processes. This communication deals with the cloning and full sequence determination of the αX subunit from CD11c/CD18 horse integrin and analyzes several structural characteristics of this molecule in comparison with those of other known species; the recognition of horse dendritic cells for mice sera against synthetic peptide designed from cloned sequence of the αX subunit of the horse integrin was also evaluated. A cDNA clone of 4582 base pairs was obtained. It encodes a protein segment of 1086 amino acid residues of the extracellular domain, a transmebrane domain of 32 residues and a C-terminal cytoplasmic tail of 24 residues. A phylogenetic analysis of this integrin shows close similarity (83%) with that of Canis familiaris (Espino-Solis, GP et al., Vet. Immunology and Immunopathology 122:326-334- 2008). In sillico analyzes by means of Swiss Prot Repostery server allowed to obtain a three dimensional model of the horse αX I-domain, from which two peptides (P1 with 20 amino acids and P2 with 17 amino acids) were chosen for chemical synthesis. The peptides were purified to homogeneity and characterized by Edman degradation and mass spectrometry analysis. They were used for production of antibodies in mice. The sera obtained from the immunized mice were characterized and used for identification of dendritic cells from horse origin. Peptide P2 was able to generate antibodies that specifically recognize dendritic cells from frozen lymphoid tissue. To the best of our knowledge these are the first antibodies that specifically recognize horse dendritic cells.

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P.049

IDENTIFICATION OF NOVEL TARGETS UNIQUE TO TUMOR-ASSOCIATED MACROPHAGES

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Recent studies have shown that tumor-associated macrophages (TAM) do not readily exert tumor-cytotoxic effects, but rather support tumor growth and metastasis. TAMs belong to the group of alternatively activated macrophages (M2) or may feature a hybrid M1/M2 differentiation. When studying several markers of non-continuous endothelial cells in murine models of transplanted malignant tumors (B16 melanoma, TS/A mammary carcinoma), we identified novel subpopulations of CD11b+ TAM co-expressing sinusoidal endothelial marker stabilin-1 or lymphatic endothelial marker LYVE-1 or both. Whereas the stabilin-1+, LYVE-1+ ,CD11b+ TAM were rare in B16 melanoma, they were the dominant TAM population TS/A mammary carcinoma.

To further characterize this TAM phenotype in general and in a tumor-type specific manner, we created an in vitro model based on bone marrow-derived macrophages stimulated with tumor-conditioned media. Expression of LYVE-1 was only induced in bone marrow-derived macrophages by stimulation with B16 melanoma-conditioned media in combination with dexamethasone and IL-4. Gene expression profiling of LYVE-1+ bone marrow-derived macrophages using Affymetrix 340 2.0 chips resulted in identifying a set of 24 highly overexpressed genes. Most of the LYVE-1 signature genes were known markers of M2 macrophages (arginase, CD163, Mgl1, -2, asf). A selection of differentially expressed molecules including a novel surface marker was confirmed in vitro and in vivo by RT-PCR, immunohistochemistry and western blotting. For the new surface molecule, specific expression in TS/A mammary carcinoma in situ was shown. Further experiments investigating the regulation and function of the protein are in progress and may help to develop macrophage-directed molecular targeted therapies for different cancers.

P.050

CONTRIBUTION OF BOTH GENETIC AND EPIGENETIC VARIABILITY TO DIFFERENTIAL GENE EXPRESSION IN MACROPHAGES FROM DISTINCT INBREED MOUSE STRAINS

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DNA-methylation is a vital epigenetic mark. It participates in establishing and maintaining chromatin structures and regulates gene transcription during mammalian development and cellular differentiation. Inter-individual differences in methylation patterns of healthy individuals have been described; however, the extent and regulatory impact of methylation differences are currently unclear. To address this issue, we have analysed methylation profiles of bone-marrow derived macrophages from two mouse strains that show marked differences in their immune responses to various pathogens (C57BL/6 & BALB/c). For example, BALB/c is susceptible to infection with intracellular pathogen Leishmania major because it develops a Th2 response. In contrast, C57BL/6 shows a Th1-type response and is resistant to L. major infection. Genetic differences are likely to contribute to the observed phenotypic differences. Whether epigentic differences, including DNA methylation, also influence the strain-specific immune reponse is currently unknown. Using expression profiling, we identified approximately 200 genes that were expressed at least five-fold different in macrophages of both strains either untreated or treated with IL-4 for 4 or 18h. Comparative methylation profiling was performed by fractionation of genomic DNA in hyper- and hypomethylated subsets using methyl-CpG-immuno precipitation (MCIp) followed by microarray hybridisation under stringent conditions and combined analysis. In total, 180 genomic regions (covering 28 Mb of the mouse genome) that were selected based on differential gene expression, were analysed for differentially methylated areas. Microarray results were independently validated using qPCR, and DNA sequencing. The combined analysis of hyper- and hypomethylated genome fractions not only allowed the identification of differentially methylated regions, but also uncovered regions that were duplicated in one strain, contained single nucleotide polymorphisms or micro- and macro-deletions. In summary, our analysis revealed a large collection of genetic and epigenetic differences in C57BL/6 & BALB/c macrophages which likely contribute to the observed phenotypic and immunological differences in both mouse strains.

P.051

Induction of Th1/Th17 immune response by Mycobacterium tuberculosis: role of cytokines produced by human dendritic cells and pathogen recognition receptors involved

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Mycobacterium tuberculosis (Mtb) subverts the functional activity of dendritic cells (DCs) and influences T cell-mediated immune responses. Here we show that Mtb alters monocytes differentiation into DCs with formation of CD14+ cells showing decreased CD1a and CD1c acquisition and producing TNF-alpha, little amounts of IL-1beta, IL-6, IL-23 and no IL-12. These cells are unable to induce substantial IFN-gamma or IL-17 production in CD4+ lymphocytes. The treatment of already differentiated monocyte-derived DC with Mtb elicits the formation of mature DC producing high amounts of IL-1beta, IL-6, IL-23, TNF-alpha, but not IL-12, and stimulating IFN-gamma and IL-17 secretion by CD4+ lymphocytes. Here we also show that IL-1beta, IL-6, IL-23 and TNF-alpha release depend on Mtb interaction with the DC receptor dectin-1, while engagement of CD206 or DC-SIGN receptor inhibits this dectin-1-dependent cytokine production. Finally, DC incubation with Mtb increased the dectin-1 expression and decreased the surface exposure of DC-SIGN and CD206. Our results indicate that Mtb modulates the functional polarization of T lymphocytes by affecting DC differentiation and cytokine release through mechanisms involving interactions of the pathogen with dectin-1, DC-SIGN and CD206 and changes of DC receptor expression. Engagement of dectin-1 by Mtb leads to an high IL-1beta, IL-6 and IL-23 production by DC, that is responsible for the Th17 response, whereas DC-SIGN and CD206 stimulation limits this process.

P.052

MODULATION OF THE INFLAMMATORY RESPONSE TO NON SELF AND SELF LIGANDS BY THE LONG PENTRAXIN PTX3

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The long pentraxin PTX3 is a multifunctional fluid-phase pattern recognition receptor, produced by innate immunity cells in response to proinflammatory signals. It is involved in innate immunity, inflammation, tissue remodelling and female fertility. PTX3 recognises non self and self ligands ranging from microbial moieties and selected pathogens to complement components, ECM components, FGF-2 and apoptotic cells. PTX3 binds OmpA, a major outer membrane protein of Gram-negative Enterobacteriaceae, which is a highly immunogenic microbial moiety, recognised by the scavenger receptors SREC and LOX1 that cooperate with TLR2 in triggering cellular responses. In the air pouch model, KpOmpA induces an inflammatory response, which is amplified by co-administration of PTX3 in terms of leukocyte recruitment and pro-inflammatory cytokine production. Experiments performed using inhibitors of the complement cascade revealed that PTX3 amplifies the inflammatory response to KpOmpA through complement activation. Moreover PTX3 has been shown to bind to apoptotic cells modulating their recognition by DC and MØ and self antigen presentation . We investigated the interaction with apoptotic cells and PTX3 and their clearance in vivo by resident peritoneal macrophages in wild type and PTX3 knock out mice. Our in vivo results indicate that phagocytosis of apoptotic cells by peritoneal F4/80+ve cells is amplified in the PTX3 knock out mice compared to wild type mice and preincubation of recombinant PTX3 with apoptotic cells significantly impaired their clearance. Thus suggesting the involvement of PTX3 in the safe removal of apoptotic cells by phagocytes. Finally, PTX3 is involved in controlling inflammation and complement activation in models of tissue damage. We are currently investigating the role of PTX3 in the inflammatory response to necrotic cells to better elucidate the role of PTX3 in discriminating self and non-self ligands.

P.053

DEFICIENCY OF THE SOLUBLE PATTERN RECOGNITION RECEPTOR PENTRAXIN-3 INCREASES SUSCEPTIBILITY TO TUMOR GROWTH AND METASTASIS

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The long pentraxin PTX3 is a multifunctional soluble pattern recognition receptor involved in innate immunity, inflammation, tissue remodelling and female fertility. Moreover PTX3 binds FGF-2 and inhibits its angiogenic activity.
PTX3 is elevated in several pathological conditions reflecting in particular the involvement of the vascular bed as ischemic heart disease, atherosclerosis, small vessel vasculitis and preeclampsia. PTX3 plasma levels are elevated in cancer patients, such as in soft tissue liposarcoma and chondrosarcoma. As PTX3 is a structural component of the cumulus oophorus extracellular matrix in human and mouse, through the interaction with hyaluronic acid binding proteins TNF-alfa-stimulated gene-6 and inter-alfa-trypsin inhibitor, we hypothesized that PTX3 could be a component of other extracellular matrices such as in the tumor stroma. Moreover, being produced by tumor stromal cells (endothelial cells, fibroblasts, leukocytes), PTX3 could play a role in modulating inflammation associated with tumor growth. To address the involvement of PTX3 in tumor growth, we used a model of non immunogenic and metastatic murine fibrosarcoma (MN/MCA1) and we observed an increased metastatic potential associated with PTX3 deficiency. Moreover in a model of immunogenic fibrosarcoma (MCA203) we observed an accelerated tumor growth in PTX3 knock out mice compared to wild type mice. Mechanisms potentially involved in the mentioned phenotypes range from regulation of angiogenesis and inflammation to organization of the extracellular matrix (ECM) through the direct interaction with ECM components and are currently under investigation. The preliminary results obtained in this study suggest that PTX3 could be a potential therapeutic target in tumor growth.

P.054

TLR4-dependent gene regulation in IL-4 versus IFN-γ primed macrophages

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Interferon (IFN)-γ and interleukin (IL)-4 are central regulators of T helper 1 (Th1) and T helper 2 (Th2) immune responses, respectively. Both cytokines have a major impact on macrophage phenotypes: IFN-γ priming and subsequent TLR4 activation induces a so called "classically activated" macrophage that is characterized by a pronounced pro-inflammatory response, whereas IL-4 stimulated macrophages, commonly called "alternatively activated", are known to develop enhanced capacity for endocytosis, antigen presentation, and tissue repair. The term "alternatively activated", however, is misleading since IL-4 is a priming rather than an activation stimulus for macrophages. Here, we investigate the TLR4 dependent gene activation program of IL-4 primed macrophages which has rarely been studied so far. Using global gene expression analysis we characterized differentially regulated genes in "classically" versus "alternatively" primed bone marrow-derived macrophages stimulated by lipopolysaccharide (LPS). We show that IL-4 primed macrophages are efficiently activated by LPS to produce a number of pro inflammatory cytokines that are generally considered hallmarks of a "classically activated" macrophage, including IL-1b, IL-6, IL-19 and IL-23. Expression of IL-12 was significantly higher in IL-4 versus IFN-γ primed macrophages and CCL17 and CCL22 transcripts were specifically induced in "alternatively primed" mouse macrophages only after LPS stimulation. In addition to chemokines and cytokines, a number of genes involved in wound healing, repair and angiogenesis were specifically induced in alternatively activated macrophages. Our data suggest that IL-4 dependent alternative priming is not per se anti-inflammatory but generates a macrophage that is capable of mounting a strong inflammatory response after TLR-dependent activation.

P.055

SECRETORY PHOSPHOLIPASES A2 INDUCE THE PRODUCTION OF ANGIOGENIC AND LYMPHANGIOGENIC FACTORS FROM HUMAN LUNG MACROPHAGES

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Angiogenesis and lymphangiogenesis are multistep phenomena critical for chronic inflammation and tumor growth and they are strictly dependent on the production of vascular endothelial growth factors (VEGFs) within the tissues. Macrophages are the predominant immune cells in normal and inflamed tissues as well as in various human tumors. A major mechanism by which macrophages influence chronic inflammation and neoplastic progression is by modulating angiogenesis and lymphangiogenesis. Secretory phospholipases A2 (sPLA2s) are enzymes released in serum and biological fluids of patients with inflammatory diseases and cancer. These molecules activate macrophages by enzymatic-dependent mechanisms or through the binding of membrane receptors such as the M-type receptor. We examined the effect of sPLA2s on the production of VEGFs (VEGF-A, VEGF-B, VEGF-C, VEGF-D and PlGF) from primary human macrophages (HLM) purified (>97%) from the lung parenchyma of patients undergoing thoracic surgery. Initial RT-PCR experiments indicated that HLM express the mRNAs specific for different isoforms of VEGF-A (VEGF-A121, VEGF-A165, and VEGF-A189), and VEGF-B (VEGF-A167, and VEGF-A186), but not PlGF. In addition, HLM express the mRNAs for VEGF-C and VEGF-D. The proteins for VEGF-A165, VEGF-B, VEGF-C and VEGF-D, but not PlGF, were detected in cell lysates by western blot. Incubation (24 h, 37°C) of HLM with increasing concentrations (0.1-10 mcg/ml) of two different sPLA2s (group IA from snake venom: svGIA; human recombinant group X: hGX) induced a significant release of VEGF-A (unstimulated: 59±16 pg/mg of protein; svGIA: 789±43, $p<0.01$; hGX: 740±32, $p<0.01$) and VEGF-C (unstimulated: 111±75 pg/mg of protein, svGIA: 527±136, $p<0.01$; hGX: 510±102, $p<0.01$). Western blot analysis of the supernatants of hGX-activated HLM showed that the VEGF-A isoform released from HLM was VEGF-A165. Quantitative RT-PCR experiments indicated that hGX induced mRNA expression of both VEGF-A165 (12.49±1.06 fold increase vs. unstimulated) and VEGF-C (8.80±1.35 fold) after 3 h of incubation. Enzymatically inactive sPLA2s (H48Q mutants of mouse and human GX) were as effective as the catalytically active sPLA2s to cause VEGF-A release. Preincubation (37°C, 30 min) of hGX with Me-Indoxam (0.1-10 mcM), which inhibits the binding of sPLA2s to the M-type receptor, concentration-dependently inhibited (IC50: 221±77nM) the release of VEGF-A induced by hGX. These results demonstrate that human macrophages express most angiogenic and lymphangiogenic factors of the VEGF family. Secretory PLA2s enhance the expression and release of VEGF-A and VEGF-C in human macrophages by a non-enzymatic mechanism involving the binding to the M-type receptor. Thus, sPLA2s may play an important role in inflammatory and/or neoplastic angiogenesis and lymphangiogenesis by inducing the production of angiogenic and lymphangiogenic factors from human macrophages.

P.056

DC-THERA DIRECTORY – A KNOWLEDGE MANAGEMENT SYSTEM SUPPORTING COLLABORATION ON DENDRITC CELL AND IMMUNOLOGY RESEARCH

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Introduction: The DC-THERA (Dendritic Cells & Novel Immunotherapies) European Network of Excellence (NoE) was established under the EU's Sixth Framework Programme. It aims at integrating the research activities of participating organisations in the field of dendritic cell (DC) immunobiology in order to develop new immunotherapies for cancer and infectious diseases. To this end, a wide range of information arising from genomics, proteomics, molecular cell biology and pre-clinical models is gathered and applied to the conduction of clinical trials.
A common problem in research carried out among different research groups distinct in location and specialisation is the organisation of the global know-how. It is also important to allow for traceability and comparability of results, methods and techniques employed.
Aims: In order to deal with these issues, we are developing the DC-THERA Directory as a comprehensive resource for accessing the DC-THERA network expertise on DC research. It includes primarily information on biological materials, protocols and data sets available within the NoE. The two main objectives of this project are to serve as a tool for collaboration among researchers in the network, and as a reference for the information contained.
Methods: We rely on the usage of standard terminologies and relations (like those defined by the Open Biomedical Ontologies project) to define the information unambiguously, to improve information retrieval and to foster interoperability. The DC-THERA KMS which follows a modelling approach inspired by technologies used in the context of the Semantic Web is based on a flexible representation schema, allowing dynamic definition of properties of biological entities, their conceptual interrelations and new types.
Results: The DC-THERA Directory provides advanced query functionalities. For instance, searching by keywords is based on ontology terms: this allows to by-pass spelling variations and to retrieve information associated to more specific terms. Results are presented with links to semantically relevant information. For instance, when displaying information on a data set, the Directory provides a description of available data, the possibility to download the data from external sources provided by the partners, a graphical and intuitive preview of data (where available) and links to related information, such as the protocols which were used to generate the data set.
Discussion: We are developing the DC-THERA Directory with the aim of providing a useful collaborative resource for the DC-THERA scientific community and an interoperable reference knowledge-base in the area of immunology and dendritic cells, for a range of activities which span from base research to clinical and pharmacological applications. The informatics infrastructure that we are building to support these goals can potentially be extended to a wider community and other know-how arising from Life Sciences-related research projects.

P.057

THERAPEUTIC POTENTIAL OF THE HUMORAL PATTERN RECOGNITION RECEPTOR PTX3 IN CHRONIC LUNG INFECTION BY PSEUDOMONAS AERUGINOSA.

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Chronic lung infections by Pseudomonas aeruginosa strains are the major cause of morbidity and mortality in Cystic Fibrosis (CF) patients, yet the molecular mechanisms leading from the mutation of cystic fibrosis transmembrane conductance regulator (CFTR) to lung infection are still unclear. Recent evidence suggest a primary defect in the immune system of CF patients leading to inability of the host to clear P. aeruginosa from the airways. PTX3 is a soluble PRR which plays non redundant roles in innate immune response to fungi, bacteria, viruses. In particular, PTX3 deficiency is associated to increased susceptibility to P. aeruginosa lung infection. Here, we address the potential therapeutic effect of PTX3 in a murine model of P. aeruginosa chronic airways infection. To this aim, we infected C57Bl/6 mice with the late clinical isolate RP73, mimicking the persistent and progressive infection occurring in CF patients and we treated mice with human recombinant PTX3. The results obtained indicate that PTX3 has a protective role in P. aeruginosa chronic lung infection, as we observed reduced lung colonization (5.8X105 CFU vs 1.6X105 CFU untreated vs treated mice respectively; p=0.01). Protection was associated with) and leucocyteβdecreased levels of pro-inflammatory cytokines (CCL2 and IL-1 recruitment in the airways (1.9X106 vs 0.7X106 total cells present in BALF, untreated and treated mice respectively; p=0.0003). Similar results were obtained in an acute model of lung infection with RP73. The mechanisms underlying the therapeutic potential of PTX3 in this model, which range from opsonization, facilitated phagocytosis, complement activation, to modulation of inflammatory responses and tissue damage, are under investigation.

P.058

THE EFFECTS OF ALPHA-1-ANTITRYPSIN ON T REGULATORY CELLS: ROLE IN ISLET TRANSPLANTATION

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T regulatory cells (Tregs) participate in immunosuppression that follows pathogenic stimuli and in preventing autoimmune diseases. Their recruitment is associated with improved allograft survival. Alpha-1-antitrypsin (AAT) is a circulating anti-protease that rises during the acute phase response and possesses anti-inflammatory properties. We recently showed that AAT decreases cell damage, pro-inflammatory cytokine secretion and immunogenicity of mouse islets in stress related conditions. In vivo, AAT therapy prolonged islet allograft survival in diabetic mice and sustained strain-specific immune tolerance that was characterized by a foxp3-positive Tregs-rich graft environment. In order to study the immunological mechanism by which AAT affects Tregs, we preformed allograft skin transplantations into various sites to permit accurate draining lymph node (DLN) collection. Allogenic skins were transplanted into hAAT treated or ALB treated transgenic mice that express GFP under the Treg specific transcription marker foxp3 (n = 13). DLN and grafts were examined for Tregs profile by direct immunofluorescent inspection, as well as IHC, FACS analysis and RT-PCR. In related studies, bone-marrow derived dendritic cells (BMDC) were treated in vitro with hAAT before stimulation with LPS, and relevant surface markers were analyzed by FACS. We observed that DLN from AAT-treated mice contained a greater population of foxp3-positive cells 4 - 7 days after transplantation, followed by increased Treg migration into grafted tissue by days 9 - 10 in AAT treated mice. Dendritic cells treated with AAT displayed lower levels of CD86 and MHC class II in vitro. In conclusion, it appears that AAT affects early events that occur after allograft transplantation. The direct effect of AAT on dendritic cell maturation may provide a mechanism for AAT-induced changes in Tregs. Our future objectives focus on identifying whether the effects of AAT on T cells are direct, or dendritic-cell-mediated.

P.059

THE FATE OF RESIDENT ALVEOLAR MACROPHAGES DURING ALLERGIC BRONCHIAL INFLAMMATION

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Introduction - Although the effector and modulator functions of activated macrophages in innate and adaptive immunity are well documented, their exact role in the initiation and propagation of immune pathologies is still not fully understood. Recent insights in monocyte and macrophage heterogeneity render the picture even more complex. In addition, it is unclear to what extend resident and elicited macrophages differ functionally and hereby differentially contribute to immune pathologies. In this study we focused on the dynamics and function of resident alveolar macrophages (rAM) during and after allergic bronchial inflammation.

Strategy - We used an ovalbumin (OVA)-alum based mouse model of allergic asthma and an OVA-CFA based mouse model of hypersensitivity pneumonitis, the Th1-driven immunological counterpart of the experimental asthma. rAM were distinguished by a prior in situ labelling with fluorescent polystyrene microspheres. As an alternative approach, rAM and elicited alveolar macrophages (eAM) were distinguished using CD45 bone marrow chimeric mice. Combined with flow cytometry and fluorescence activated cell sorting, both approaches allowed us to trace both AM populations in the course of a Th1- and Th2-driven allergic airway inflammation.

Results - During the acute phase of the allergic response, isolated rAM and eAM showed a distinct gene expression signature, reflecting a possible functional heterogeneity between these two macrophage subsets. In both types of allergic bronchial inflammation, microsphere-tagged CD45.1+ rAM remained constant in cell number for the first 2 days of chronic OVA-exposure and then dropped sharply, having nearly completely disappeared from the alveoli by day 4 of OVA-exposure. Preliminary experiments in experimental asthma showed the presence of a significant caspase-activity in rAM by day 2 and 3 of OVA-exposure. A possible mechanism for the rapid decline of rAM-numbers during sustained allergic inflammation therefore is a caspase initiated apoptotic event. The near complete disappearance of the rAM has as a consequence that, following the clearance of inflammation; a renewed population of macrophages resides in the airways. Strikingly, in both types of allergic inflammation, this 'secondary' rAM population showed a marked alteration in transcriptional reactivity to LPS stimulation. Especially because the type of allergic inflammation (Th1- vs. Th2-mediated) seems to influence the basal gene signature and LPS-reactivity of 'secondary' rAM, the functional differences implicated, might represent an 'innate memory' of the preceding inflammatory insult.

P.060

CIRCULATING TUMOR MARKER-CONTAINING MACROPHAGES: A PROMISING NEW TOOL WITH POSSIBLE CLINICAL IMPLICATIONS

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Introduction: The mechanisms responsible for recruiting monocytes from the bloodstream into solid tumors are now well characterized. From histological examinations it is known that these cells then differentiate into macrophages. One of the functions of these macrophages is the clearance of tumor remnants by phagocytosis.

Hypothesis: After fulfilling its function, the macrophage returns to the bloodstream and will go to the reticulo-endothelial system for its own clearance. The detection of tumor remnant-containing activated macrophages in peripheral blood creates a possibility to detect even low levels of tumor-related particles due to the intracellular concentration effect. A trial was conducted in patients with different types of cancer.

Materials & Methods: Mononuclear cells were collected from peripheral EDTA-blood samples of patients with carcinomas of the prostate or the colorectal tract. After labeling the activated macrophages by membranous staining with CD14-APC/CD16-FITC, phagocytized tumor-remnants were detected by incubating the cells with an rPE-conjugated anti-tumor marker (e.g. PSA-rPE or CEA-rPE) using a fix&perm strategy. The labeled cells were subsequently flowcytometrically analyzed. Also peripheral blood from matched control-patients as well as from patients with benign prostate and benign colorectal diseases were analyzed.

Results: In both cancer groups (prostate as well as colorectal) a significant difference was found in the size of the fraction of PSA- or CEA-containing activated macrophages, respectively, as compared to the group of patients with benign disorders. In prostate cancer, there was even a significant difference between patients with a benign prostatic hyperplasia and localized prostate cancer.

The fraction of CEA-containing macrophages showed significant differences ($p<0,001$) between all the malignant groups (AJCC-stage II to IV) and the control group. The AJCC-group I showed a significance level of $p<0,05$ ($p=0,001$) with the healthy control group.

Conclusion: The fraction of tumor marker-containing activated macrophages can be determined in prostate and colorectal cancer patients. This new assay provides a method to detect malignant disease in earlier stages than the standard serum assay, and can also be used for monitoring patients during therapeutic interventions. Recently performed pilot studies have already revealed that this concept can also be adapted to other types of tumor marker-related particles as well as to other types of cancer.

P.061

FAP induces DC maturation through NF-kB dependent pathway

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Fibronectin attachment protein (FAP) are a family of fibronectin binding proteins present in several species of mycobacteria. Studies targeting FAP as an inducer of immunity in mycobacterial infections suggest that FAP is a highly immunogenic protein, but little is known about the protective immune response to this microorganism. Dendritic cells (DC) are the professional antigen-presenting cells responsible for initiating of the immune response. In present study, we analyzed interaction between FAP and DC to characterize the role of this protein in promoting innate and adaptive immune responses. FAP functionally activates bone marrow-derived DC by augmenting expression of the surface markers, CD40, B7 family (CD80 and CD86) and major histocompatibility complex class I and II. FAP induces production of Th1-promoting interleukin-12 from DC and augments the allogeneic immunostimulatory capacity of DC. FAP stimulates production of interferon-gamma from T cells in mixed lymphocyte reactions, which suggesting Th1-polarizing capacity. CD4+ T cells stimulated by FAP-stimulated DC show a Th1-polarizing cytokine profile. The expression of surface markers on DC is B pathways. Ourκmediated by both mitogen-activated protein kinases and NF- findings suggest that FAP induces maturation of DC and drives Th1 polarization, which are important properties for determining the nature of immune response against M paratuberculosis.

P.062

DPT induces the dendritic cells maturation: Implication of dendritic cells-based tumor vaccination adjuvant

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DPT a medicinal herbal product isolated from Anthriscus sylvestris. Anthriscus sylvestris is a perennial herb that grows in Europe, Caucasus, Siberia, China, Manchuria, and Korea. The root of this plant has been used in Korean traditional medicine as an antitussive, an antipyretic, an analgesic, a diuretic, and a cough remedy. This plant has been demonstrated to possess a lignan, deoxypodophyllotoxin which is known to have antitumor activity. Dendritic cells (DC) play significant role in the development and maintenance of anti-tumor immune response. The effects of DPT on DC are remaining unknown. DPT induced surface molecule expression, cytokine production, endocytosis capacity, and underlying signaling pathways in murine bone marrow-derived DC. The dendritic cells were then assessed with regard to surface molecule expression, dextran-FITC uptake, cytokine production, capacity to induce T cell differentiation, and underlying signaling pathways. DPT was shown to significantly increased CD80, CD86, MHC class I, and MHC class II expression in production. Theγimmature DC. The DC also increased IL-12 expression and IFN- DPT-treated dendritic cells was found to be highly efficient in regards to Ag capture via mannose receptor-mediated endocytosis. DPT was also demonstrated to induce MAPK activation and NF-κB nuclear translocation. Moreover, the DPT treated DC manifested induction of Th1 responses, and normal cell-mediated immune responses. In a therapeutic model of murine melanoma, DPT-pulsed DC significantly retarded tumor growth and improved the survival of tumor-bearing mice.

P.063

P-glycoprotein is essential for dendritic cells differentiation, development and T cells polarization

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Dendritic cells (DC) are professional antigen-presenting cells that have the ability to sense infection, simple and present antigen to T lymphocytes, and represent a bridge between innate and adaptive immunity. DC expresses the ATP-binding cassette transporters P-glycoprotein (P-gp). P-gp is a 170-kDa transmembrane protein encoded by the mdr-1 gene, a member of a highly conserved superfamily of ATP-binding cassette transport proteins, plays, in addition to its ability to efflux toxins, a role in the resistance to pathogensexpressed on various tissues, including peripheral blood lymphocytes and DC. Functionally, P-gp transpoters have been described to be required for efficient DC and T cells migration. Recently, P-gp has been found to be expressed in monocyte-derived DC and interstitial DC and migration toward lymph nodes via afferent lymphatic vessels. In human skin explants system, it was described that Abs or drugs that antagonize P-pg such as verapamil block this migration. In this study, we found that P-gp is also crucial for DC differentiation and development. Down-regulation of P-gp inhibits the differentiation of DC and cytokine levels during DC maturation. Moreover, the P-gp-inhibited DC manifested impaired production. Theseγinduction of T cell polarizations, proliferation, and IFN- results indicated that P-gp is a critical role for differentiation of DC and DC-mediated immune responses.

P.064

MINCLE, THE MACROPHAGE-INDUCIBLE C-TYPE LECTIN, PLAYS A FUNDAMENTAL ROLE IN THE INNATE IMMUNE RESPONSE AGAINST Candida albicans

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Efficient detection of pathogens by the cells of the innate immune system is the first essential step for combating infection. This recognition is based on a complex network of interacting receptors; the C-type lectins are emerging as key contributors to this process. Here we describe a novel C-type lectin, Mincle, whose deficiency rendered mice susceptible to infection with C. albicans. We present evidence that Mincle has a non-redundant role in recognition and signaling in response to the yeast.

Mincle is a 219 amino acid type II transmembrane protein with a highly conserved C-type lectin domain, whose carbohydrate ligand has not yet been identified. We have used a Mincle gene knock-out mouse line to characterise its role in a natural infection model of the yeast C. albicans. In vivo, Mincle knock-out mutants were significantly more susceptible to the yeast in both systemic and oral infection. In vitro, Mincle deficient bone marrow derived macrophages demonstrated normal phagocytosis of C. albicans. However, cytokine production was attenuated and, in particular, TNF-alpha production upon stimulation with C. albicans was dramatically lower in Mincle knock-out macrophages than in wild type cells.

Immunofluorescence studies in mouse tissue sections showed Mincle is present on the cell surface of resident macrophages, with particular abundance in the alveolar macrophages of the lung. Besides, real-time PCR analysis showed widespread expression of the Mincle gene, suggesting that the presence of this receptor is needed throughout the organism.

The recognition of carbohydrate moieties on pathogens is emerging as an important modifier of immune outcomes. Our results establish a fundamental function of Mincle in C. albicans recognition and demonstrate its requirement for the induction of an inflammatory immune response.

P.065

DISTINCT IMPAIRMENT IN THE EARLY ACTIVATION OF TYK2 DEFICIENT PERITONEAL MACROPHAGES AND SPLENOCYTES UPON ENDOTOXIN CHALLENGE

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Septic shock, caused by the Gram-negative bacterial cell wall component lipopolysaccharide (LPS), is a widely feared complication in modern intensive care units. Multiple organ failure is caused by an overreaction of the immune system with dysregulated cytokine production.

We and others reported previously, that mice deficient for Tyk2 are not susceptible to high dose LPS shock and show diminished responses to IL-12, IL-18 and interferons (IFN). In addition, they have defects in LPS induced IFN production.

We therefore wanted to clarify if the impaired immune response in Tyk2-/- mice is correlated with a decrease in the activation status of immune cells upon stimulation with LPS.

LPS stimulated peritoneal macrophages (PMs) and spleen cells from LPS treated mice were analysed by flow cytometry. Cells were stained with CD69, to examine the early activation and with CD86 to check the capacity of T-cell co-stimulation. The number of positive cells and the relative increase of cell surface molecule density on positively gated cells were compared among different genotypes.

In contrast to Ifnar1-/- PMs, which showed no increase in CD69+ cells, there was a clear upregulation of CD69+ cells in Tyk2-/- PMs, but the absolute number of positive cells was considerably lower than in wild-type.

Upon LPS challenge in spleen derived T-cells, dendritic cells, natural killer cells and macrophages an increase of CD69+ cells was observed for all genotypes. But compared to wild-type a significant reduction in the total number of CD69+ cells was shown for Tyk2-/- and Ifnar1-/- to a similar extent. In contrast, in both mutants the number of CD69+ B-cells was unaffected.

Regarding the T-cell co-stimulatory molecule CD86, there was no effect of Tyk2 in PMs and splenic B-cells. Ifnar1-/- B-cells were also wild-type while PMs lacking Ifnar1 showed no increase of CD86+.

In summary, Tyk2-/- PMs and most spleen cells were significantly impaired in their early activation upon LPS stimulation as shown by the markedly decreased number of CD69+ cells. A complete dependency of the LPS response on Ifnar1 was only observed in PMs.

P.066

Chondroitin sulfate disaccharide stimulates microglia to adopt a novel regulatory phenotype

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A disaccharide degradation product of chondrotin sulfate proteoglycan-disaccharide (CSPG-DS) has been implicated previously in the inhibition of neurodegeneration by influencing microglia activation. In this study, genome-wide microarray
analysis was used to identify specific gene expression profiles of CSPG-DS-stimulated BV-2 microglia-like cells. Gene products involved in phagocytosis, detoxification, migration, immune regulation, and antigen presentation were found to
be altered significantly. These findings were replicated and compared with IFN-γ-stimulated primary microglia using real-time quantitative RTPCR validation. Importantly, a unique transcriptional phenotype with anti-inflammatory and IFN-γ counter-regulatory properties partially related to alternatively activated macrophages was identified.
Using functional cell assays, we found that CSPGDS-stimulated microglia possess increased phagocytic capacity but lack direct cytotoxic effects such as secretion of NO. Furthermore, conditioned media from CSPG-DS-treated microglia did not diminish the viability or cause apoptosis of cultured photoreceptor cells and partially rescued these cells from IFN-γ-induced apoptosis. Taken together, our data provide a unique transcript dataset and important in vitro findings about the functional properties of CSPG-DS-activated microglia. These might be starting points to explore the in vivo role of CSPG-DS as a bioactive microglia regulator and its potential, therapeutic application in immune-related, neurodegenerative disorders.

P.067

Standardized determination of CD14+CD16+ and CD14++ monocyte numbers as a monitoring system

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The two main subpopulations of human blood monocytes are defined as CD14 strongly positive, CD16 negative (CD14++CD16-) and CD14 weakly positive, CD16 positive (CD14+CD16+). We here report on a staining protocol for determination of the absolute number of these monocyte subsets using a single platform approach with CountBrightTM absolute counting beads and four colour flow cytometry with the fluorescent–labelled antibodies CD14 My4 FITC, CD16 3G8 PE, HLA-DR PC5 and CD45 APC. With this assay we are able to efficiently exclude natural killer cells and granulocytes into the CD14+CD16+ monocyte gate. For the CD14+CD16+ monocytes the intra-assay CV was 5.64 % and the inter-assay CV was 13.56 %. Looking at a cohort of 50 donors aged 18 to 60 years we did not find any age dependence.
Using this novel approach we can confirm that exercise (400 W, 1 min) will lead to > 3-fold increase of the CD14+CD16+ monocytes. Also, we show that therapy with glucocorticoids will deplete these cells.
This robust single platform assay may be a useful protocol for monitoring monocyte sub-populations with high precision in health and disease.

P.068

INFLAMMATORY MONOCYTES PROTECT SHIP-DEFICIENT MICE FROM EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Experimental autoimmune encephalomyelitis (EAE) is a neuroimmune model that mimics many aspects of human multiple sclerosis (MS) - an inflammatory disease of the central nervous system (CNS) mediated by myelin-primed CD4(+) T cells. Although the onset of EAE is an adaptive immune response, innate immune cells such as CNS-resident microglia, peripheral macrophages, granulocytes, as well as other innate immune cells recruited to the CNS, participate in the cycle of neurological damage and repair that propagates the disease. However, the specific contributions of these innate immune cells in the context of autoimmunity are poorly understood.
Shortly after T cell priming, during induction of EAE in mice, a population of myeloid-derived suppressor cells with a CD11b(+)Ly6G(-)Ly6C(high) surface antigen phenotype is increased in the spleen and circulation. These inflammatory monocytes (IMC) up-regulate NO synthase 2 (iNOS) and produce nitric oxide (NO) in response to interferon-gamma to suppress activated T cells. IMC demonstrate functional plasticity since, in response to interleukin-4, they up-regulate arginase 1 (Arg1) and down-regulate iNOS and thus may lose their suppressive phenotype. The SH2-containing inositol 5'-phosphatase, SHIP, is a negative regulator of the PI3-kinase pathway in hematopoietic cells. Deletion of SHIP leads to an altered immune environment such that peritoneal and alveolar macrophages in SHIP-/- mice have an M2-skewed "healer" phenotype in contrast to classically activated "killer" macrophages (M1). M2 macrophages express constitutively high levels of Arg1 and produce low levels of NO. We hypothesized that the M2-skewed environment and hyper-responsive myeloid phenotype of SHIP-deficient mice would enhance disease progression in the EAE mouse model. Instead, we found that SHIP-deficient mice are protected from EAE with delayed onset of disease symptoms and a reduced clinical score. Although some SHIP-/- mice (but none of the wild-type litter mates) fail to show disease symptoms, most of these mice become symptomatic. Thus, deletion of SHIP does not block antigen-presentation altogether. We have attributed disease protection to a greater number of CD11b(+) cells in the spleen of naïve SHIP-/- mice and enhanced induction of Ly6C(high) inflammatory monocytes following antigen-priming. Antigen-specific recall response and proliferation of splenocytes (and lymph node cells) from SHIP-deficient mice are suppressed compared to wild-type controls. We conclude that the suppressor phenotype of IMC in the M2-skewed environment of SHIP-/- mice is retained. We plan to adoptively transfer antigen-primed T cells or activated IMC from SHIP-deficient mice into naïve wild-type recipients and to use lineage-specific Cre/Lox SHIP-deletion models to further define the functions of these cell populations in the propagation of EAE.

P.069

CD34-DERIVED MYELOID DENDRITIC CELL DEVELOPMENT REQUIRES INTACT PI3K-PKB-mTOR SIGNALING

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Dendritic cells (DC) comprise different subsets with specific functions in the induction and regulation of immune responses. The myeloid subsets include Langerhans cells (LC) and interstitial DC (intDC). The molecular mechanisms regulating DC survival, differentiation and proliferation are largely unknown. Most studies on human DC are performed with monocyte-derived DC (moDC), the counterparts of inflammation driven intDC in vivo. We previously reported that GM-CSF-induced mTOR activity is crucial for the survival of both developing and fully differentiated moDC. As opposed to the inflammatory moDC, CD34-derived DC may better resemble DC continuously developing during the steady state. Here, we investigated the importance of PI3K-PKB-mTOR signaling in CD34-derived LC and intDC. Human CD34+ cord blood-derived hematopoietic progenitors were cultured with GM-CSF, TNFα and SCF. CD14-CD1a+Langerin+ LC and CD14-CD1a+ intDC emerged within 2 weeks via precursors that could be distinguished and FACSorted at day 6 by their respective CD1a+CD14- and CD1a-CD14+ phenotypes. Inhibition of either PI3K (2μM LY294002; LY) or its downstream effector mTOR (20nM rapamycin; Rapa) severely hampered DC development. LY and Rapa dramatically reduced the CD1a+ DC yield at 14 days (2.42+/-0.89 and 6.66+/-3.6 fold respectively; $p<0.01$). The reduced DC yield was partly caused by a decreased cell cycling, as demonstrated by an increased (10%) G0/1 phase. However, the main effect was due to the induction of apoptosis (up to 30% reduced viable AV-PI- cells at all time points). Cell viability was increased with 15% upon introduction of constitutively active PKB (myrPKB) by retroviral transduction. LY-, but not Rapa-induced apoptosis was blocked in these cells, confirming that PI3K-induced mTOR activation was mediated via PKB. Interestingly, more differentiated cells were less sensitive. Since differentiation was not affected by either inhibitor, cultures in the presence of LY or Rapa resulted in a relative but not absolute enrichment of CD1a+ intDC and Langerin+ LC. In accordance with this observation, survival of terminally differentiated DC was not affected. Also LPS-induced maturation was unchanged. In line with the in vitro data, preliminary results show increased human DC numbers in β2 microglobulin-/- NOD/SCID mice transplanted with human CD34+ cells expressing myrPKB. In conclusion, PI3K-PKB-mTOR signaling is crucial for both proliferation and survival of developing CD34+-derived myeloid DC. In contrast to moDC, which acquire LY/Rapa sensitivity during development, the PI3K/mTOR dependence of CD34-derived DC decreases during development with terminally differentiated DC being unaffected by LY/Rapa. These data not only show that signaling pathways responsible for DC survival change during differentiation, but also indicate that, depending on the physiological conditions leading to their development, myeloid DC subtypes use different signaling pathways for their survival.

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CCL2 DOWN-MODULATION TRIGGERED BY SELECTED TOLL-LIKE RECEPTOR AGONIST COMBINATIONS CONTRIBUTES TO TH1 POLARIZING PROGRAM IN HUMAN DENDRITIC CELLS

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Toll-like receptors (TLRs) sense microbial products and induce the coordinated activation of innate and adaptive immune responses by activating dendritic cells (DCs). Full activation often requires synergistic stimulation triggered through pairs of different cell surface receptors, providing the host with a mechanism to react rapidly and specifically to different pathogens. We describe herein that simultaneous activation of TLR4 and TLR8 signaling cascades in human monocyte-derived DCs (MD-DCs) results in a marked inhibition of CCL2 secretion and mRNA accumulation as well as of NF-kB p65 binding activity with respect to that observed in cells stimulated with single agonists. Inhibition of CCL2 is specific for both CCL2 and TLR agonist combination with LPS exhibiting the features of a dominant suppressor factor. CCL2 production triggered via TLR engagement is mediated by type I IFN but it is not negatively affected by IL-10. Interestingly, down-modulation of CCL2 parallels a concomitant up-regulation of IL-12 production and it is required for an optimal Th1 polarization and IFN-γ production by T lymphocytes. Overall, these results indicate that CCL2 supplies an important immunomodulatory role to DCs and that the modulatory effect of this chemokine may dictate the cytokine profile in Th1 responses induced by DCs.

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P.071

ERYTHROPOIETIN ATTENUATES MACROPHAGE EFFECTOR FUNCTIONS

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Erythropoietin (EPO) is commonly used in the treatment of anemia including the anemia of inflammation (AI), which occurs in a broad range of diseases characterized by immune activation including malignancies, autoimmune disorders and infections. We investigated the putative modulatory effects of EPO on the innate immune response in models of Salmonella infection. We report that EPO attenuates macrophage effector functions both in vitro and in vivo, presumably by reducing the binding affinity of nuclear factor (NF)-κB. This regulatory effect inhibits the expression of pro-inflammatory mediators including TNF, IL-6 and Mcp-1, thus impairing pathogen clearance and exacerbating Salmonella infection. Our data indicate that in the setting of AI, EPO must be used with caution considering the etiology of immune activation because administration of this cytokine may be fatal in acute infections.

P.072

SHIP REGULATES TLR-INDUCED ACTIVATION OF Flt3L- AND GM-CSF-DERIVED DENDRITIC CELLS

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Dendritic cells (DCs) are a migratory group of bone marrow derived leukocytes with a specialized role in the uptake, transport, processing and presentation of antigens to T cells. In the presence of molecules indicative of danger, DCs undergo maturation (marked by upregulating co-stimulatory molecules CD80, CD86 and CD40), increase their cytokine (eg IL-12 and TNFα) production and migrate to lymph nodes where they present MHC-bound antigenic peptides to T cells. The maturation of DCs is highly pathogen-specific and the intracellular pathways stimulated by different Toll like receptors (TLRs) also instruct DCs to initiate either a TH1 or TH2 response in T cells. In part this is accomplished by different signalling pathways downstream of the TLRs, which can be broadly classified as either MyD88-dependent or MyD88-independent. MyD88-dependent signalling (eg. TLR 9) promotes rapid activation of NFκB and induction of pro-inflammatory cytokines, while MyD88-independent signaling (eg. TLR3) mediates slow activation of NFκB and induction of IFN-inducible genes and co-stimulatory molecules. One pathway that has been shown to modulate NFκB-induced DC maturation and activation is the phosphatidylinositol 3-kinase (PI3K) pathway. This pathway is negatively regulated by the SH2 containing inositol 5'-phosphatase, SHIP, via the hydrolysis of the second messenger PIP3 to PI-3,4-P2. However, the precise role of SHIP in innate activation of DCs has not been determined. Using both GM-CSF- and Flt3L- derived DCs we have investigated how SHIP-deficiency affects their ability to respond to innate immune activation and induce T cell proliferation. We have found that the role of SHIP not only varies depending on the TLR agonist used but also by the derivation condition. In Flt3L-derived DCs, SHIP negatively regulates both pro- and anti-inflammatory cytokine production when stimulated with TLR-agonists that employ MyD88-independent signalling pathways. In addition, maturation and DC-induced T cell proliferation is attenuated, but TH1 skewing is enhanced, in SHIP-deficient DCs but only if the TLR ligand acts via a MyD88-independent pathway. On the other hand GM-CSF-derived SHIP-deficient DCs have altered cytokine production relative to wild type DCs, but the differences do not depend on the TLR agonist used. SHIP-deficient GM-CSF DCs have reduced maturation as well as the corresponding reduction in T cell proliferation and TH1 skewing. These findings illustrate variable roles for PI3K and SHIP in DCs derived in different conditions and downstream of specific TLR ligands.

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THE MUCOSAL ADJUVANT LTK63 DIRECTLY ACTIVATES IMMATURE DENDRITIC CELLS AND INCREASES THE NUMBER OF FUNCTIONALLY ACTIVE DENDRITIC CELLS IN THE LUNGS OF MICE

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LTK63, a detoxified mutant of the E. coli heat labile enterotoxin (LT), is a potent mucosal adjuvant and was shown to induce generic protection. Dendritic cells are likely targets of adjuvants, but the primary target cell of LTK63 is unknown. Therefore, we aimed to investigate the effect of LTK63 in vitro on murine BM-DCs and we evaluated ex vivo the effects of intrapulmonary LTK63 administration on cell numbers, phenotype and function of DCs in the mouse lung. The first observation was that unsorted BM-DCs cultured in the presence of GM-CSF for 4 and 6 days are responsive to LTK63, while BM-DCs generated for 8 days are unresponsive, suggesting that DC precursors or very early DCs are direct target cells. To test this directly, FACS sorted MHC-II(low) immature DCs and MHC-II(high) mature DCs were treated with LTK63. Only immature DCs upregulate CD86 and CD80, but not CD40 or MHC-II after LTK63 treatment. At difference to LPS or CpG, which induce a wide range of cytokines, LTK63 induces the production only of IL-6, IL-12, KC and RANTES by immature DCs. In contrast, production of IL-1b, MIP-1a and MCP-1 is inhibited by LTK63. We conclude from this data that LTK63 directly activates MHC-II(low) immature DCs and that this property may be the basis of the adjuvant effect of LTK63. Studies are underway to confirm immature DCs as target cells of LTK63 also in vivo in the lung. When LTK63 was administered in vivo, we observed two phases of response in the lung: an early phase, which extends from 1 to 2 days, and a second phase, which extends from 6 to 8 days. In the first phase, LTK63 induces up-regulation of IL-1b, G-CSF and KC, and the increase of the number of MHC-II(high) mature mDCs and granulocytes. In the second phase, at 6-8 days, LTK63 induces a strong increase of the number of mature mDCs, pDCs and other cell types and up-regulation of a complex mixture of cytokines involved in inflammation and cell recruitment. Using an in vivo migration assay, we found that at least part of the LTK63-induced mDC accumulation at 6 to 8 days is due to the recruitment of differentiated DCs from the blood. The total population of CD11c+ cells present at 8 days after LTK63 treatment is more efficient than those from buffer-treated mice at stimulating allogeneic T cell responses. This is due to the numerical increase of CD11c+ MHC-II(high) DCs with a higher allostimulatory activity than CD11c(high) MHC-II(int) cells. We conclude that immature mDCs are a direct target of LTK63 and that adjuvanticity and generic protection induced by LTK63 in vivo are linked to increased numbers of CD11c+ MHC-II(high) mDCs with a strong potential to prime T cells.

P.074

LACTOFERRIN, A MAJOR DEFENSE PROTEIN OF INNATE IMMUNITY, IS A NOVEL MATURATION FACTOR FOR HUMAN DENDRITIC CELLS

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Lactoferrin (LF) is an important protein component of the innate immune system that is broadly distributed within the body fluids. LF is endowed with multiple biological activities. Talactoferrin a recombinant human LF is in clinical development as an anti-cancer agent and is entering Phase III clinical trials. Here we show that TLF induces the maturation of human DCs derived from monocytes. Talactoferrin, at physiologically relevant concentrations (100 µg/ml) up-regulates the expression of HLA class II, CD83, CD80 and CD86 costimulatory molecule and CXCR4 and CCR7 chemokine receptors, acting primarily through the p38MAPK signaling pathway. DCs matured by talactoferrin displayed an enhanced release of IL-8 and CXCL10, and a significantly reduced production of IL-6, IL-10, and CCL20. They also display a reduced ability to take up antigen, and increased capacity to trigger proliferation and release IFNg in the presence of allogeneic human T cells. Talactoferrin-matured DC are able to prime naïve T cells to respond to KLH antigen and display a significantly increased capacity to present Flu-MA58-66 peptide to HLA-A2 matched T cells.
These data suggest that a key immunomodulatory function that may be mediated by talactoferrin is to link the innate with adaptive immunity through DC maturation.

P.075

Involvement of dendritic cell subsets during Cyclophospamide-induced immune activation: implications for the design of combination therapy protocols against cancer

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Many clinical studies based on the combination of chemotherapy and other anticancer treatments have been published over the years showing variable responses. This therapeutic approach, has been considered to be inappropriate for years based on the belief that the efficacy of cancer vaccines would be inhibited by the immunosuppressive effects of chemotherapy. Recently, new knowledge has been generated on the immunomodulatory properties of some chemotherapeutic agents, such as cyclophosphamide (CTX), leading to a renewed interest in combination therapy regimens for cancer. In previous studies we observed that CTX can exert, on one hand, a direct effect on the tumor mass leading to the control of tumor growth and, on the other hand, an immunostimulatory activity through the modulation of the expression of various soluble factors (cytokine storm). Since the induction of an effective antitumor response requires the active participation of host APCs responsible for adequate antigen presentation and lymphocyte priming, we investigated on the effects of CTX treatment on dendritic cells (DCs) in vivo. We show that in mice implanted with EG7.OVA thymoma, CTX treatment induced a transient reduction of total bone marrow cells, but not of DC precursors, which, instead, proliferate displaying enhanced DC generation capabilities in vitro. Accordingly, in secondary lymphoid organs, conventional CD8α+ DCs, the key DC subset specialized in the cross-presentation of cell-associated antigens, undergo a transient and selective depletion followed by a rebound phase, in a way similar to what previously described for lymphocytes. In addition, plasmacytoid DCs, the main type I IFN producers, progressively accumulate in the spleen and in the lymph nodes of CTX-treated tumor-bearing mice. Current studies are aimed at clarifying the function and the role of the different DC subsets during chemotherapy, with the final aim of optimizing combination therapy protocols against malignancies.

P.076

DEFECTIVE T CELL EDUCATION BY DENDRITIC CELLS CONTRIBUTES TO IMPAIRED ANTIGEN-SPECIFIC IMMUNITY IN WISKOTT-ALDRICH SYNDROME

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Normal immunity is critically dependent on the spatial and temporal distribution of immune cells. Dendritic cells (DC) prime antigen-specific T cells in secondary lymphoid tissue and stimulate them to exert their effector function. Wiskott-Aldrich Syndrome protein (WASp) is an important cytoskeletal regulator and expressed exclusively in immune cells. Reduced or absent expression of WASp as a result of mutations gives rise to Wiskott-Aldrich Syndrome (WAS), an X-linked immunodeficiency. At cell level, WASp deficiency (WAS KO) causes defective cytoskeletal rearrangement resulting in impaired podosome formation and reduced motility of DC. We have shown previously that impaired migration of WAS KO DC results in reduction of WASp-competent T cell priming in vivo. In addition, WAS KO DC were intrinsically less effective in priming T cells in vitro. This led us to investigate the role of WASp in DC mediated T cell education. WAS KO DC demonstrated reduced ability to induce proliferation of allogeneic Balb/c CD4+ T cells and antigen-specific OT2 CD4+ T cells. When the interaction of ovalbumin-pulsed DC with OT2 T cells was analysed using real time imaging, WAS KO DC formed less stable clusters with OT2 T cells as determined by the average duration of a cluster and the number of times clusters disaggregated and were re-formed. Additionally, WAS KO DC induced reduced calcium signalling in OT2 T cells after DC-mediated cognate interaction, and confocal microscopy revealed a smaller immune synapse size and impaired polarization of T cells towards WAS KO DC. Collectively these findings indicate that WASp-deficient DC are less effective at establishing a stable immune synapse that is required for optimal antigen presentation and specific T cell stimulation. This likely contributes significantly to immune deficiency in WAS and suggests that curative therapies for WAS, such as bone marrow transplant and gene therapy, should aim for correction of both myeloid and lymphoid lineages.

P.077

TOLL-LIKE RECEPTORS DIFFER IN THEIR ACTIVATION OF NF-KAPPAB: IMPLICATIONS FOR IL-12P40 INDUCTION

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Interleukin-12 is a pro-inflammatory cytokine produced by antigen-presenting cells. It serves an important role in the differentiation of T-cells towards TH1 phenotype. Various TLR agonists induce IL-12 expression to differing extend with high IL-12 induction observed after TLR9 stimulation, intermediate IL12 induction after TLR4 stimulation and weak IL12 induction observed after TLR2/6/1 stimulation. Mice lacking IL-12 have a strong defect in cell mediated immune responses resulting in a high susceptibility to infections with intracellular pathogens including Leishmania major, Listeria monocytogenes or Toxoplasma gondii. On the other hand exaggerated expression of Th1-inducing cytokines may contribute to the development and perpetuation of chronic inflammatory and autoimmune diseases. Aim of the present study was to investigate the mechanisms involved in differential IL-12p40 induction upon stimulation with different TLR ligands. Using pharmacological inhibitor and different knockout mice models MAP kinase, PI3 kinase and IRF signaling pathways could be ruled out to be the cause for the differences in IL-12p40 induction in bone marrow derived dendritic (BMDC). Stimulation of BMDC with different TLR ligands resulted in striking differences in the activation kinetics of the NFkappaB pathway with early NFkappaB activation observed after LPS (TLR4) stimulation and a delayed NFkappaB activation after CpG (TLR9) stimulation. These differences in NFkappaB activation resulted in rapid but short-lived Rel-A IL-12p40 promoter recruitment after LPS stimulation and prolonged Rel-A binding at the IL-12p40 promoter after CpG stimulation. Only TLR2 and TLR4 ligands were capable to induce serine 536 phosphorylation of Rel-A which was responsible for early termination of NFkappaB activation at the IL-12p40 promoter. Evidence will be given that differences in activation of NFkappaB are involved in the differential IL-12p40 induction after stimulation with the various TLR agonists.

P.078

GLUCOCORTICOIDS INDUCE REGULATORY MONOCYTES THAT INFLUENCE T CELL RESPONSES

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Glucocorticoids (GC) are still the most widely used immunosuppressive agents in clinical medicine. Surprisingly little is known about the mechanisms of GC action on monocytes which play a central role in propagation as well as resolution of inflammation. In a murine model we show that Glucocorticoids (GC) promote survival of anti-inflammatory monocytes that influence T cell responses in antigen- specific and unspecific ways. Thus, GC-treatment does not lead to global suppression of monocytic effector functions it rather leads to induction and differentiation of monocytes to become regulatory cells. Using gene chip and protein expression analysis we show that GC- induced regulatory monocytes up-regulate surface molecules like CD163, and CD124 while others e.g. CCR2 and CX3CR1 are down- regulated. Upon GC- treatment transmigration and motility of these cells is up-regulated while adherence is reduced. Furthermore, co-culture of regulatory monocytes and T cells leads to inhibition of T cell activation as shown by diminished proliferation and cytokine production in antigen-dependent and antigen- independent ways. We examined the mechanisms of regulatory function of monocytes, and show that they regulate T cell activation independent of IL-10 that is induced on RNA level by GC in regulatory monocytes. In addition we determined cell-cell contact dependent inhibition of T cell responses. In conclusion, GC treatment generates regulatory monocytes that are capable to control ongoing T cell responses, and thus have a high potential to become valuable tools in immunotherapy against inflammatory diseases.

P.079

TLR AGONIST R848 INHIBITS DIFFERENTIATION OF mDCs AND LEADS TO THE DIFFERENTIATION OF TOLEROGENIC APC FROM CD14+ MONOCYTES

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We have shown previously that TLR agonists interfere with the GMCSF and IL4 (G4) driven differentiation of immature mDC from CD14+ monocytes in vitro. Among the TLR agonists the TLR7/8 agonist R848 is the strongest inhibitor of mDC differentiation. Stimulation of CD14+ monocytes in the presence of G4 and R848 resulted within 5 days in a cell type that is characterized by an increased PDL-1 and a decreased CD1a expression. The induced deviant APCs preferentially induce CD25+ FoxP3+ T regulatory cells. We have analysed here the mechanisms of action of R848 on DC differentiation. Therefore we determined cytokine secretion and MAP-kinases activation in R848 and G4 stimulated monocytes. In comparison to G4 stimulated monocytes, R848 plus G4 stimulated monocytes released extraordinary high IL-6 amounts and less TNF, IL-10 and IL-12p40. Kinetic analyses revealed that stimulation with R848 for 24h was sufficient to prevent mDC differentiation and likewise to allow induction of deviant APC. Peak of cytokine release was within the first 24 h of stimulation. In some experiments blockade of IL-6 was able to prevent the inhibition of CD1a induction, suggesting that IL-6 signal transduction might be important in preventing CD1a upregulation and in inducing deviant APC. Analyses of MAPK activation pattern during stimulation of monocytes revealed differences. Stimulation R848 plus G4 resulted in a stronger p38 phosphorylation as compared to G4 alone. P44/42 phosphorylation was higher with R848 and G4 within the first 15 min. However, at later time points p44/42 phosphorylation was lower. The most striking changes were found with JNK. Upon stimulation with R848 and G4 a strong phosphorylation was observed which was not seen with G4 alone. These results show that concomitant TLR stimulation during G4-driven differentiation of monocytes leads to a deviant APC phenotype. Differential induced cytokines as well as differential triggered MAP-kinases seem to mediate this deviation of differentiation. During infection this pathway may be exploited by pathogens to modulate immune responses.

P.080

IKKβ MAINTAINS THE PHENOTYPE OF TUMOUR-ASSOCIATED MACROPHAGES

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Within most human and murine cancers there is an abundant macrophage population, attracted to the tumour microenvironment by cytokines and chemokines. Tumour-associated macrophages (TAM) are obligate partners for malignant cell migration, invasion and metastasis. Some molecular insights into the role of TAM have come from papers published in the past two years showing that selective ablation of NF-kappaB signalling in myeloid cell populations inhibited the development of experimental inflammation-induced cancers of the intestine. We hypothesized that inhibiting NF-kappaB signaling in TAM will switch their phenotype and render these macrophages cytotoxic and immuno-activating. The specific ablation of NF-kappaB signalling in macrophages alters their phenotype, rendering them cytotoxic for malignant cells. We adoptively transferred NF-kappaB signalling-inhibited macrophages to mice with established peritoneal ovarian cancer. This adoptive transfer led to a significant reduction in tumour growth. The decrease in tumour growth corresponds with an increased IL-12 and decreased IL-10 and TNF-alpha production. Furthermore, after transfer of the modified macrophages, cell surface Class II MHC expression increases on the entire peritoneal macrophage population. We conclude that NF-kappaB signaling is involved in maintaining the phenotype of tumour-associated macrophages and that inhibition of this pathway is capable of reverting their pro-tumour functions to an anti-tumour cytotoxic role. This approach provides a complimentary therapeutical option to render the immunosuppressive tumour microenvironment.

P.081

IMPLICATION OF LIVER X RECEPTORS (LXRs) IN THE RESOLUTION OF INFLAMMATION AND AUTOIMMUNITY

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The Liver X Receptors (LXRs) are members of the nuclear receptor superfamily of transcription factors that play central roles in the transcriptional control of lipid metabolism. Activation of LXRs promotes the expression of genes involved in cholesterol homeostasis and inhibits the expression of inflamatory genes in metabolic and immune cells, such as macrophages. In addition, LXR signaling is important for the innate immune response against listeria monocytogenes infection. Thus, the study of LXR function in macrophages is unraveling previously unrecognized links between innate immunity and lipid metabolism.

Chronic inflammation and disregulation of the immune mechanisms are currently underlying many human diseases. While essential to combat pathogens, macrophage-derived cytokines are also injurious to normal cells and tissues. If unchecked at the resolution of immune responses, production of cytokines leads to chronic inflammation and autoimmunity. For example, systemic lupus erythematosus (SLE) is a chronic inflammatory disease with unknown etiology in which the immune system turns its defenses upon self-elements such as chromatin. SLE is characterized by lymphocyte expansions, hypergammaglobulinemia and renal damage.

We will describe our initial studies with mice lacking LXRs, which manifest an age-dependent breakdown in self-tolerance and develop a lupus-like disease. We will also discuss the potential implications of LXR-dependent pathways in the protection against autoimmune disease and the potential mechanisms involved. Our results demonstrate that LXRs play an important role in the protection against autoimmune disease and suggest that pharmacological activation of LXR could be a therapeutic alternative to ameliorate inflammatory disregulation during autoimmune disease and other inflammatory disorders.

P.082

A differential distribution of a functional polymorphism in the HLA-E gene in Sardinia suggests a possible role of Natural Killer function in Ankylosing Spondylitis pathogenesis.

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Ankylosing spondylitis (AS) is a rheumatic disorder that is strongly associated with HLA–B27. However, HLA–B*2709, an allele that occurs in high frequency among the population of Sardinia, is not associated with the disease and has been found within a haplotype different from that harboring the associated B*2705 allele. This leaves open the possibility that other genes mapping in the same haplotype could contribute to the lack of association. The strong linkage disequilibrium present in the HLA region makes it difficult to single out genes with possibly weak influence in the

presence of the major susceptibility factor HLA–B27. In this context, fine mapping of the HLA region, comparing the haplotypes harboring B*2705 and B*2709, may provide insight. The Sardinian population is an ancient genetic isolate with a unique distribution of alleles at multiple loci and is therefore particularly appropriate for association studies. A previous study in which the allelic frequencies of the classic HLA class I and II genes were analyzed showed no major differences between AS patients and B*2705-matched controls, while allowing identification of an extended B*2709 haplotype (HLA–A32;Cw1;B*2709;DR5). 117 patients with AS, 111 HLA–B27–positive controls (of whom 31 were positive for HLA–B*2709), and 214 randomly selected controls were genotyped for microsatellites and single-nucleotide polymorphisms (SNPs) spanning the HLA region. Haplotypes carrying either the B*2705 or the B*2709 allele were found to share a conserved region downstream of the HLA B gene and a functional polymorphism in the HLA–E gene (R128G), while differing in all other markers. Notably, the presence of an A at SNP rs1264457, encoding for Arg-128, was significantly ($P < 0.0001$) increased in the cohort of patients but not in B*2705- or B*2709-positive controls. Comparing the alleles co-occurring at each HLA marker, we identified a region differentiating patients with AS and B*2705-matched controls. In particular, there was a markedly increased prevalence of heterozygosity at rs1264457 among B27-positive controls suggesting a protective role of G128 in AS. Moreover, other markers around the HLA–B gene were also differentially represented. These results demonstrate a significant difference in the frequency of some HLA markers between AS patients and B*2705-positive controls, which could be attributed to the opposite chromosome. In particular, the differential distribution of a functional polymorphism in the HLA–E gene suggests a possible role of natural killer function in AS pathogenesis.

P.083

Modulation of Dendritic Cells by Mycobacterium avium ssp.

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Mycobacterium avium subspecies (ssp.) paratuberculosis (MAP) is the etiological agent of paratuberculosis, a chronic, non-treatable granulomatous enteritis of ruminants. MAP is the only mycobacterium affecting the intestinal tract. This is of interest since it is presently the most favoured pathogen linked to Crohn´s disease (CD) in humans, in which MAP was detected often in affected intestinal tissues samples. MAP is genetically closely related to other Mycobacterium avium ssp. (MAA) which in contrast to MAP can cause mycobacteriosis in animals and immunocompromised humans. Following oral ingestion MAP translocates from the intestinal lumen into the intestinal tissue via M-cells of the domes of Peyer´s patches. After crossing the epithelial barrier of the intestine MAP persists and multiplies in intraepithelial macrophages. The factors that aid to MAP tropism and progression of inflammation in the gut as well its interaction with dendritic cells (DC) are still unclear. We have recently shown that murine macrophages represent a suitable system to analyse Mycobacterium avium ssp. patho-mechanisms. In this study we investigated the interaction of MAP with murine dendritic cells. For this, bone marrow derived murine DCs were infected with different multiplicities of infection (MOI) of viable and heat killed MAP and MAA. Both ssp. induced DC maturation as monitored by CD86 and MHCII expression, independently on viability and the infection dose. Expression of pro- and anti-inflammatory cytokines (TNF, IL-12, IL-10) was examined by RT-PCR and ELISA. These factors were dose dependently induced by MAP and MAA, however independently on viability, indicating that heat stable mycobacterial components contribute to their induction. Taken together, the results indicate that both MAP and MAA activate DCs independently of their viability. Further experiments are necessary to examine if DCs contribute to MAP induced progression of inflammation in the gut.

P.084

HERPES SIMPLEX VIRUS TYPE I (HSV-1) REPLICATES IN MATURE DENDRITIC CELLS AND CAN BE TRANSFERRED IN A CELL-CELL-CONTACT DEPENDENT MANNER

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Herpes simplex virus type I (HSV-1) is a very successful representative of the alpha-herpesvirus family and about 90 percent of the population are seropositive for this particular virus. While the pathogen usually causes the well known mild lesions on the lips, also severe infections of the eye or even the brain can be observed in rare cases. It is well known, that this virus can efficiently infect the most potent antigen presenting cell i.e. the dendritic cell (DC), both in its immature and its mature state. While the infection of the immature DC has been shown to be productive, infection of mature DC (mDC) is believed to be abortive in the early phase of the viral replication cycle. In line with these findings, no virus particles can be detected in the supernatant of HSV-1 infected mDC. In this study, however, we show for the first time that besides the reported immune evasion strategies used by the virus e.g. downmodulation of CCR7 and CD83 or interfering with TAP and the STAT1 pathway, this pathogen completes its replication cycle in mDC. We detected the presence of viral gene transcripts of all three phases of the replication cycle as well as of late viral proteins and even the generation of progeny virus. While we could confirm the findings, that these particles are not released to the supernatant, surprisingly the newly generated viral particles can be passed on to permissive Vero cells in a cell-cell-contact dependent manner, but not when the infected mDC were physically separated from the permissive cells. Finally, we provide evidence, that the viral glycoprotein E (gE) is involved in the transfer of infectious virus from mDC to other permissive cells.

P.085

mDCAR1 - Characterization of a novel member of the C-type lectin family

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The murine dendritic cell (DC) immuno-receptors (mDCIR) and DC immuno-activating receptors (mDCAR) represent a subfamily of cell surface C-type lectin (CLEC) proteins, whose multifunctional capacity range from classical antigen uptake and immuno-regulatory mechanisms to the involvement in DC ontogeny. Here, we describe a novel member of the DCIR/DCAR family named mDCAR1. Following the generation of specific monoclonal antibodies (mAb) against mDCAR1, extensive phenotypical characterization identified a heterogeneous CD11b+Gr-1+/dimF4/80+CD117dimCD115dim progenitor population of the early granulocyte and monocyte lineage in bone marrow and spleen. While in spleen mDCAR1 is further expressed on CD8alpha+ DC, receptor expression in thymus is restricted to these DC. No expression could be found in blood or lymph nodes. With respect to the classical function of CLEC as antigen uptake receptors, the selective expression pattern and observed endocytic activity following receptor crosslink suggests that it can be used for antigen targeting to DC.
Triggering of mDCAR1 with specific mAb initiates signal transduction resulting in tyrosine phosphorylation of proteins and preliminary results further indicate a regulation of Toll-like receptor (TLR)-induced cytokine secretion. Through the identification and characterization of mDCAR1 as a member of the CLEC family, the knowledge on CLEC will be further extended being crucial to understand their importance for and to assess their multiple functions in immune interactions.

P.086

PRIMING AND CROSS-PRIMING COMPETENCE OF PLASMACYTOID DENDRITIC CELLS AFTER ANTIGEN-UPTAKE VIA mPDCA-1

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Plasmacytoid Dendritic cells (PDCs) are a distinct subset of dendritic cells and have a central role as sentinels for pathogens. PDCs produce large amounts of interferons upon microbial stimulation and are believed to link innate and adaptive immune responses. However, their exact function as antigen-presenting cells for the initiation of adaptive immune responses is controversially discussed and it remains unclear whether PDC are in fact able to prime naïve T cells. In this study we investigated the function of the recently described PDC-specific receptor mPDCA-1. Cross-linking of mPDCA-1 with specific monoclonal antibody (mAb) (JF05-1C2) resulted in a rapid internalization of the mAb-receptor-complex both in vitro and in vivo. Targeting of PDCs with OVA-conjugated anti-mPDCA-1 mAb, but not with an equivalent amount of soluble OVA or OVA conjugated to isotype control antibody, resulted in strong proliferation of OVA-specific naïve CD4+ T cells from OVA-TCRtg mice. PDCs were also able to cross-present mPDCA-1-targeted OVA protein to naïve CD8+ T cells from OVA-TCRtg mice. Again, mPDCA-1-mediated antigen uptake was more efficient in inducing a strong T cell response compared to unspecific uptake. Blocking the receptor with excess of unconjugated anti-mPDCA-1 mAb inhibited priming of both CD4+ and CD8+ T cells. These results indicate that mPDCA-1 may serve as an antigen uptake receptor delivering its ligands for MHC-I and MHC-II presentation.
Interestingly, processing and presentation of antigens by PDCs after antigen-uptake via mPDCA-1 was strongly dependent on TLR stimulation, since only activated but not immature PDCs were able to prime naïve antigen-specific CD4+ and CD8+ T cells. In contrast, antigen uptake was independent on activation as unstimulated PDC also internalized the mAb-receptor-complex.
Our results demonstrate that PDCs take up and process antigens for efficient priming of CD4+ and CD8+ T cells and thus combine innate and adaptive functions.

P.087

Genome-wide location of PU.1 and C/EBPbeta binding sites in human monocytes and macrophages

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Human monocyte to macrophage differentiation is accompanied by pronounced phenotypical changes and generally proceeds in the absence of proliferation. The molecular events governing this process are poorly understood. Previously, we have shown that C/EBPbeta and PU.1 were involved in regulating macrophage-specific gene expression. To further elaborate the role of both transcription factors in macrophage differentiation, we have generated high-resolution maps for the genome-wide location of C/EBPbeta and PU.1 binding sites in human monocytes and monocyte-derived macrophages using ChIP-sequencing technology. Whereas PU.1 binding is relativley stable in both cell types, the number of C/EBPbeta binding sites strongly increases during differentiation. Both transcription factors are frequently found together and bound at proximal promoters and even more pronounced at sequences upstream- and downstream of gene promoters. An integrated analysis of ChIP-Seq data, motif search and whole genome expression data will allow the identification of cell-type and differentiation-specifc roles for both transcription factors. This study provides a framework for further investigations on cell-type specific gene expression in human monocytes and macrophages.

P.088

pUL128 OF HUMAN CYTOMEGALOVIRUS (HCMV) - A NEW CHEMOKINE EXPRESSED ON THE SURFACE OF VIRAL PARTICLES?

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Human cytomegalovirus is a widespread opportunistic pathogen affecting children in utero and immunocompromised patients. Monocytes are considered to be primary targets, site of latency and vehicles for HCMV dissemination in vivo. Previously, we have shown that TB40E, a HCMV virus strain widely used for infection of endothelial cells, infects monocytes and impairs their chemokine-responsiveness by a mechanism that is independent on viral gene expression. By Ch. Sinzger we obtained two Bacterial Artificial Chromosome (BAC) clones, BAC4 and BAC1, derived from TB40E and we investigated their phenotype in primary human monocytes. While the clone BAC4 exhibited the same phenotype as TB40E to infect and block the migration of monocytes, the clone BAC1 did not. Though the BAC1 viral particles were able to enter monocytes, we could not detect de novo viral gene expression and the chemokine responsiveness of monocytes was normal. The genetic comparison of BAC4 and BAC1 (Sinzger et.al, J Gen Virol. 2008 Feb;89:359-68) revealed one coding-relevant nucleotide insertion in the UL128 region, leading to a truncated form of pUL128 in BAC1-infected cells. Interestingly, it has been published that pUL128 possesses four conserved cysteine residues characteristic of CC- chemokines and that it is located on the outer surface of the viral particles where it forms a complex with gH, gL, UL130 and UL131 that defines infectivity and cell tropism. To prove the involvement of pUL128 in this phenotype, we compared the migratory properties of monocytes treated with a mutated BAC4 (BAC4mut) and a repaired BAC1 (BAC1rep), expressing the truncated form or the full-length form of the pUL128, respectively. We observed that BAC4 and BAC1rep, the strains expressing full-length pUL128, were able to block the migration while monocytes infected with BAC1 and BAC4mut, variants expressing the truncated protein, migrated normally. We confirmed the direct role of pUL128 in inhibition of chemotaxis, performing migration assays with monocytes treated with different doses of a recombinant UL128 protein (rpUL128). A dose of 1µg/ml of rpUL128 was sufficient to induce the same block of migration that was induced by BAC4 and BAC1rep. The inhibitory effects was not due to a LPS contamination of the recombinant protein and was specific for rpUL128, since monocytes treated with an irrelevant recombinant protein migrated normally. When used as chemoattractant, rpUL128 induced monocyte recruitment with the same potency as MCP-1. All together, these observations lead us to the hypothesis that the virally surface protein UL128 could be a new viral encoded chemokine.

P.089

UP-REGULATION OF PRX5 IN IMMUNOSTIMULATED MACROPHAGES

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Macrophages are involved in many facets of innate immunity and are well equipped to produce harmful anti-microbial products including reactive oxygen species and nitric oxide (NO). Release of NO and O2-, when produced concomitantly and in close vicinity, yields peroxynitrite, a strong oxidant which is thought to represent an effective weapon against intracellular pathogens. Oxidative stress induces adaptive mechanisms of cytoprotection, and along this line, we undertook a study to determine the regulation of Prx5, a potent peroxide and peroxynitrite reductase, in macrophages. Murine bone marrow-derived macrophages (BMM) were activated by IFN-γ or lipopolysaccharide (LPS). Minute amount of either IFN-γ or LPS increased Prx5 expression at mRNA and protein level. When added in combination, the stimuli barely further increased Prx5 protein level suggesting redundancy of IFN-γ and LPS pathways. Using NOS2 KO mice, we showed that NOS2-derived NO is not involved in Prx5 up-regulation. We also showed that LPS/TLR4-dependent increase in Prx5 expression is mediated by a MyD88-independent/TRIF-dependent/IFN-β-independent pathway. Unsurprisingly, IFN-γ-dependent induction of Prx5 gene was independent of TLR4 and TRIF but interestingly, was markedly reduced in MyDD88-/- and TNF-/- BMM. Overall, we suggest that Prx5, by contributing to the control of redox homeostasis, helps keeping activated macrophages alive and potent. In this way, it can be proposed adding Prx5 to the components of the cytotoxic armature of activated macrophages.

P.090

DIFFERENTIAL IRF-3/IRF-7 ACTIVATION IN PDC AFTER CSFV INFECTION: IMPLICATIONS FOR PATHOGENESIS

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Classical swine fever virus (CSFV) induces the proteosomal degradation of interferon regulatory factor 3 (IRF-3) by means of the viral protein Npro. As a consequence IFN-a/b responses are blocked in CSFV-infected porcine cell lines, macrophages and conventional dendritic cells (DC) but not in plasmacytoid DC (pDC), in which IFN-a/b is induced through activation of IRF-7 early after infection e.g 2-8 hours post-infection (PI). Despite this, our data indicate an important contribution of IRF-3 to pDC responses, as mutant CSFV with dysfunctional Npro produced significantly larger quantities of IFN-a/b when compared to wildtype (wt) CSFV. We demonstrate that in pDC, similar to non-pDC, IRF3 is rapidly degraded by wt CSFV (10 hours PI) but not by Npro mutant CSFV. While after infection with wt CSFV the initial IRF7 activation is followed by a "natural" IRF-7 degradation, in pDC infected with the Npro mutant virus a prolonged IRF-7 expression was observed which was reflected by significantly higher IFN-a production. Based on IFN-b blocking studies, we propose that an early IRF-3 mediated IFN-b production in pDC acts as a positive feedback loop for IFN-a secretion in CSFV-infected pDC. Accordingly, IFN-b treated pDC produce larger quantities of IFN-a after stimulation with CpG. Surprisingly, both wt and mutant CSFV were highly virulent and induce aberrant high levels of serum IFN-a/b in infected pigs. It seems that a complex balance between Npro-mediated inhibition of IFN-a/b production and virus replication-dependent induction of IFN-a in pDC plays an important role in CSF pathogenesis.

P.091

ROLE OF COMPLEMENT COMPONENT C3 IN THE DIFFERENTIATION AND FUNCTION OF HUMAN DENDRITIC CELL

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Complement is known as one of the major components of natural immunity. It's effector functions include opsonization, phagocytosis and lysis of various microbes. The deposition of C3 fragments allows the binding of antigens to cells expressing different complement receptors (CR). CR3 and CR4, members of the beta2-integrin family are expressed on several cell types, including dendritic cells (DC). The most important role of these receptors is the binding and phagocytosis of pathogens opsonized by C3-fragments.

Since immune complexes occuring in vivo contain C3 fragments, as well, our aim was to study whether they influence maturation and different functions of monocyte-derived DCs (MDCs). Dendritic cells (DCs) link innate and adaptive immunity by their ability to present antigens to naive T lymphocytes.

In our present experiments immature monocyte-derived DCs (imMDCs) were generated in vitro from human monocytes in the presence of rhuIL-4 and rhuGM-CSF. The cells were treated with purified human C3 or with normal human serum (NHS) and were further cultured for two days. Binding of C3-fragments to MDCs was detected by cytofluorimetry and confocal microscopy. The maturation of MDCs was characterized by the expression of various cell membrane molecules using cytofluorimetry. As positive control, LPS was added to the cells (maMDCs). As a result of treatment with C3 or NHS enhanced CD83, CD86 and MHCII expression was detected, while the level of MR did not change. The functional activity of MRs of C3-treated MDCs was checked by phagocytosis of FITC-dextran. The capacity of C3 or NHS-treated MDCs to activate allogeneic T lymphocytes was also assessed by 3H-thymidine incorporation. We found, that C3- or NHS- treated MDCs strongly stimulated the proliferation of T cells. Proliferation of T cells was significantly reduced when the MDCs were pretreated with C3 specific antibody. Our further aim to characterize the role of C3 in cytokine production of MDCs . The production of cytokines IL-6, IL-8 and TNF-α are also increased by C3-treated MDCs. We modelled the near in vivo situation, where macrophages and DCs developed from the same donor were cocultured. We found that activated Ms can opsonize adjacent MDCs with C3-fragments initiating their maturation. Activated Ms locally opsonize the particles in their microenvironment with C3 thereby enhancing their phagocytosis by MDCs.

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THE MANNOSE RECEPTOR AS A PUTATIVE HEPATITIS B VIRUS RECEPTOR REGULATING INTRAHEPATIC DENDRITIC CELL FUNCTION

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Worldwide over 350 million people are chronically infected with the Hepatitis B virus (HBV). Chronic HBV infection is the result of an inadequate anti-viral immune response. Dendritic cells (DC) of chronic HBV patients are reported to be dysfunctional. Since DC play a major role in anti-viral immunity, impaired DC function upon HBV infection could contribute to HBV persistence. Recently, we showed that myeloid DC (mDC) display an impaired function after exposure to HBV surface antigen (HBsAg) in vitro. To search for possible interference of HBV with DC function in vivo and the molecular mechanisms involved, we investigated the HBsAg internalization route and the presence of HBsAg-positive mDC in chronic HBV patients in vivo. HBsAg (1 ug/ml) uptake and lectin expression were studied with isolated CD1c+ peripheral blood-derived mDC from healthy controls. The presence of HBsAg and the expression of mannose receptor (MR) on peripheral blood and percutaneous needle liver biopsy-derived mDC from chronic HBV patients (n=8) were investigated by (intracellular) flow cytometry after staining blood/liver-derived mononuclear cells for HBsAg and MR. In vitro uptake of HBsAg was hardly detectable in freshly isolated peripheral blood-derived mDC, but increased to 27±2% HBsAg+ mDC during 18 h of culture. The glycosylation pattern of HBsAg pointed towards a possible role for C-type lectins expressed on mDC in its uptake. Analysis of C-type lectin expression on freshly isolated and cultured mDC by RT-PCR and flow cytometry revealed a significant upregulation of MR upon culture. Both the mannose-specific lectin inhibitor mannan and a neutralizing anti-MR antibody reduced the HBsAg-uptake by mDC with 50%. Then, the MR as possible receptor involved in HBsAg uptake by mDC in vivo was studied in peripheral blood- and liver-derived mDC of chronic HBV patients. Flow cytometric analysis showed that mDC circulating in peripheral blood of HBV patients do not express the mannose receptor, while liver biopsies derived from the same patient group showed 20±7% MR+ mDC. Strikingly, also significantly more HBsAg-positive mDC were detected in liver biopsies compared to blood (6.7±2.5 vs 0.68±0.3% HBsAg+ mDC; p= 0.0078). In conclusion, these data show a role for the mannose receptor in HBsAg uptake by mDC both in vitro and in vivo. The presence of HBsAg in intrahepatic mDC, together with the immune regulatory role of HBsAg on mDC as shown before, suggests that HBV actively interferes with DC function. This immune regulatory role of HBV may well contribute to HBV persistence.

P.093

CHEMOKINE EXPRESSION PATTERNS IN PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA

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Chemokines are small proteins known to function as regulatory molecules in leukocyte maturation, traffic and homing, as well as in development of lymphoid tissues. Numerous chemokines have been identified as attractants of different type of blood leukocytes to sites of infection and inflammation. A vast, ever-increasing literature documents the expression of multiple chemokines in tumours and of chemokine receptors by tumour cells. Nevertheless, it is still matter of debate the function of chemokines in tumour develop and growth.
We have hypothesized that homeostatic and inflammatory chemokines, which control cell trafficking in secondary lymphoid organs (CXCL13, CXCL12, CCL21), might play a crucial role in the development, progression, and impaired immune response observed in extranodal lymphomas, and in particular in primary CNS lymphoma (PCNSL). Immunohistochemical analysis and in situ hybridization on 25 cases of PCNSL revealed a high protein expression of CXCL12, which can also function as survival factor for malignant B cells. In addition, we have characterized the cells of the immune system within the tumour microenvironment and assess the expression of selected inflammatory chemokines. The in vitro analysis of chemokine responses induced in a cell line derived from a diffuse large B cell lymphoma of activated phenotype (OCI Ly10) shows that the interplay of inflammatory and homeostatic chemokines present in the tumour microenvironment can strongly influence the migratory capacity of malignant B cells. Chemokine expression patterns of malignant lymphocytes, vascular endothelium, and infiltrating lymphocytes might influence tumour development, localisation, as well as the inflammatory response in the microenvironment.

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HUMAN CYTOMEGALOVIRUS INFECTION ALTERS THE M1-M2 POLARIZATION OF HUMAN MACROPHAGES

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Macrophages play an important role in host defence and in the maintenance of tissue homeostasis. After infection by human cytomegalovirus (HCMV) they support viral replication and secrete a plethora of soluble factors. Since it has become clear that macrophages may display even opposite functions depending on their activation state, we investigated the effects exerted by HCMV on the polarization of monocyte-derived macrophages (MDM). Starting form circulating monocytes we obtained subsets of pro- and anti-inflammatory MDM by using the lineage-determining cytokines GM-CSF or M-CSF, respectively (as described by Werreck F.A.W. PNAS 2004). Pro- and anti-inflammatory macrophages, designated M1 and M2, significantly differed for the surface expression of CCR1, CD1a, CD80, CD206, MHC-II, CD163 and CD36. Moreover, M1 macrophages exhibited higher basal and IL-1. We first observed that HCMV infectionαsecretion levels of TNF- differed in the two macrophage types. While the infection was rapidly lytic and productive in M2 macrophages, M1 macrophages were resistant to infection and produced lower amounts of progeny virus. We characterized the phenotype of HCMV-infected macrophages by FACS analysis and ELISA measurements of M1 (IL-12, , IL-8, Rantes) or M2 (IL-10, IL-1ra,β and α, IL-6, IP-10, MIP-1β, IL-1αTNF- eotaxin) soluble mediators. While HCMV-infected M1 cells did not show changes in the expression of surface molecules as compared to mock-infected cells, HCMV-infected M2 macrophages underwent down-regulation of CCR1 and CD36 and up-regulation of CD80. Additionally, in both types of macrophages, HCMV andαinfection led to a massive release of M1 cytokines, such as IL-12, TNF- IL-1. Surprisingly, both M1- and M2 cells also secreted in the supernatants high levels of M2 cytokines, such as IL-10, IL-1ra and eotaxin after infection. The atypical "activation" state of HCMV-infected macrophages was also reflected by their incapacity to stimulate the proliferation and activation of lymphocytes, as measured by MLR.

In summary, our data reveal that HCMV interferes with the normal M1-M2 polarization of macrophages inducing a mixed phenotype where pro- and anti-inflammatory features coexist.

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A NETWORK OF PROTEIN INTERACTIONS IN MACROPHAGES IMPLICATES LYSOSOMAL BIOLOGY AND THE COMPLEMENT PATHWAY IN ATHEROGENESIS

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Cholesteryl ester accumulation by macrophages promotes foam cell formation, a key event in atherogenesis. The impact of lipoproteins on the delivery and removal of cholesterol to and from macrophages has been widely studied. However, macrophages are of central importance in immunity, apoptosis, and metabolism, and investigations of genetically engineered mice have implicated each of these pathways in atherogenesis. It is thus likely that broad networks of interacting proteins and genes, rather than simple linear pathways that affect only sterol balance, are involved in foam cell formation and atherogenesis. To begin to develop an integrated view of the macrophage foam cell, we used tandem mass spectrometry to analyze the shed and secreted proteome of cultured macrophages. Global analysis of the 542 proteins detected with high confidence in macrophage-conditioned medium revealed a single large network of 505 interactions among 238 proteins. Rigorous statistical analysis identified 15 proteins that were differentially expressed by foam cells. A sub-network centered on those proteins linked cholesterol deposition in macrophages to 3 functional modules: lysosomal biology, lipid metabolism, and complement regulation. Three lines of evidence support the proposal that this sub-network is important in atherogenesis. First, over one-third of the proteins that mapped to the sub-network associated with atherosclerosis phenotypes in genetically engineered mice. Second, proteomic analysis showed that an overlapping but distinct set of proteins in the same network were differentially secreted by macrophage foam cells isolated from Apoe-/- mice, a genetic model of hypercholesterolemia. Third, this altered protein expression pattern was attenuated by treating Apoe-/- mice with simvastatin, a therapy which retards atherosclerosis without affecting plasma lipid levels. Collectively, our observations provide an integrated view of the macrophage foam cell, linking functional modules centered on immunity and lipid metabolism in macrophages with atherosclerosis susceptibility.

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ANALYSIS OF THE EXPRESSION OF THE NEW MYELOID MARKER PTPg ON HUMAN LEUKOCYTES BY MULTIPLE FLUORESCENCE FLOW CYTOMETRY

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PTPRG (PTPg), a receptor-like protein tyrosine phosphatase located in 3p14.2, is considered an oncosuppressor gene and is thought to have a role in haematopoietic differentiation. We recently reported the development of an antibody raised in chicken against an epitope located in the extracellular domain of PTPg. This antibody has proven to recognize the protein in its native and denatured form and was the first being used for flow cytometry of purified peripheral blood leukocytes without the need of fixation and permeabilization. Following this first analysis, this time we set up a multiple stainings panel for the analysis of PTPg expression in peripheral blood white cells subtypes starting from whole blood and using selected lineage and activation markers.The analysis was performed in 5 normal subjects. In the lymphoid population, CD4+ and CD8+ T cells were negative according to previous data. The same conclusions also apply to NK cells, with no apparent differences between CD16+/CD56dim and CD16-/CD56bright cells. Similarly, there was no difference in PTPg expression between CD8+ and CD8- NK cells. B cells were the only lymphocytes showing a clear expression of PTPg. In the myeloid compartment, Neutrophils confirmed to express a low amount of PTPg in about 15% of the expression in monocytes and population. We also confirmed high levels of PTPg CD1c+ dendritic cells, with higher expression within CD83+/CD1c+ subpopulation. No expression was detected in the small subtype of CD141+ dendritic cells, known to define a very rare (0.02%) subset of human dendritic cells called MDC2. CD303+ Plasmacytoid dendritic cells showed three distinct populations according to the expression of CD86 and PTPg; CD86-/PTPg- (85%), CD86+/PTPg- (10%) and CD86-/PTPg+ (5%), being this last population CD14dim. Taken together, these data provide the first detailed analysis of PTPg expression in leukocytes, a necessary step for further studies aimed to the analysis of PTPg modulation in health and disease.

P.097

Hypoxia Transcriptionally Induces CCL-20 In Bp50κPrimary Human Monocytes Through NF-

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Hypoxia is a condition of low oxygen tension occurring in inflammatory tissues that creates a special microenvironment conditioning cell physiology. Peripheral blood monocytes (Mn) represent the early mononuclear phagocyte infiltrate in hypoxic tissues. We investigated the gene expression pattern of primary human Mn following exposure to hypoxia (1% O2) and identified a group of novel hypoxia-responsive genes related to the inflammatory responses. Among a ,)/CCL20α (MIP-3αthem, we studied the macrophage inflammatory protein-3 CC-chemokine selectively chemotactic for immature DC, activated/memory T lymphocytes, and naive B cells. Hypoxic upregulation of CCL20 was demonstrated in vitro both in Mn and Mn-derived macrophages and confirmed in vivo in Mn infiltrating the inflamed synovia of patients affected by Juvenile Idiopatic Arthritis. mRNA induction was paralleled by increased protein expression and secretion and dependent on gene transcription activation. Functional studies of the CCL20 promoter using a series of 5′-deleted and mutated reporter constructs B binding motif located at positionκdemonstrated the requirement for the NF- –92/–82 for gene transactivation by hypoxia, because: (i) transcription was B motif; (ii) three copies of theκabrogated by a 3 bp mutation of the NF- B binding site conferred hypoxia responsiveness to a minimalκwild-type NF- B binding to thisκheterologous promoter; (iii) hypoxia increased specific NF- Bp50κsequence. Furthermore, we provided evidence of a role for the single NF- family member in mediating CCL20 gene transcription in hypoxic Mn. p50 B-siteκB complexes binding the cognate κhomodimers were the only detectable NF- Bp50 knockdown byκon the CCL20 promoter upon hypoxia exposure, and NF- lentiviral-mediated shRNA interference resulted in binding inhibition, whereas Bp50 in transient cotransfection studies promoted CCL20κoverexpression of NF- B-site.κgene transactivation, which was abrogated by mutation of the –92/–82 Finally, we demonstrated a regulatory role for the hypoxia-inducible factor rapidly induced in hypoxic Mn, in NF-kB-driven CCL20 upregulation. In ,α(HIF)-1 conclusion, this study characterizes a previously unrecognized role for hypoxia as a transcriptional inducer of CCL20 in human Mn and highlights a functional interplay between the NF-kB and HIF pathways in mediating this response, with potential implications for inflammatory disease and cancer pathogenesis.

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Transcriptome of Hypoxic Immature Dendritic Cells: Modulation of Chemokine/Receptor Expression

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Hypoxia is a condition of low oxygen tension occurring in inflammatory tissues. Dendritic cells (DC) are professional antigen-presenting cells whose differentiation, migration, and activities are intrinsically linked to the microenvironment. DCs will home and migrate through pathologic tissues before reaching their final destination in the lymph node. We studied the differentiation of human monocytes into immature DCs (iDCs) in a hypoxic microenvironment. We generated iDC
in vitro under normoxic (iDCs) or hypoxic (Hi-DCs) conditions and examined the hypoxia-responsive element in the promoter, gene expression, and biochemical KEGG pathways. Hi-DCs had an interesting phenotype represented by up-regulation of genes associated with cell movement/migration. In addition, the Hi-DC cytokine/receptor pathway showed a dichotomy between down-regulated chemokines and up-regulated chemokine receptor mRNA expression.
We showed that CCR3, CX3CR1, and CCR2 are hypoxia-inducible genes and that CCL18, CCL23, CCL26, CCL24, and CCL14 are inhibited by hypoxia. A strong chemotactic response to CCR2 and CXCR4 agonists distinguished Hi-DCs from iDCs at a functional level.
The hypoxic microenvironment promotes the differentiation of Hi-DCs, which differs from iDCs for gene expression profile and function. The most prominent characteristic of Hi-DCs is the expression of a mobility/migratory rather than inflammatory phenotype. We speculate that Hi-DCs have the tendency to leave the hypoxic tissue and follow the chemokine gradient toward normoxic areas where they can mature and contribute to the inflammatory process.

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Polarization of MTOC, cytokines and polarity proteins in dendritic cells during synapse with naïve CD8+ T cells.

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Dendritic cells engage in immune synapse (IS) with T cells during the initiation of adaptive immunity. This cell-cell interaction facilitates molecular communication between membrane proteins like MHC peptide complexes/TCR and co-stimulatory molecules. T cell activation within the IS is accompanied by a clear assimetric distribution of cytoskeletal and signalling proteins that reorients towards the APC. The molecular events that link TCR signaling to T cell polarity have been characterized quite extensively. In contrast little is known about polarization events occurring in DCs during synapse formation. Here we have investigated cell polarity in DC forming conjugates with naïve CD8+ T cells. We found that rapidly after conjugates formation DC reorient the microtubule-organizing center (MTOC) toward the T cell in an antigen dose dependent manner. In DCs deficient for the Wiskott-Aldrich syndrome protein, a key regulator of cytoskeletal dynamics, MTOC reorientation is dramatically reduced. Proteins associated to establishment of polarity in other cell systems (like Disc Large proteins DLG and scribble) are expressed in DCs and are highly enriched at the region of antigen specific interaction with T cells, in close proximity to MTOC. To investigate the functional meaning of cell polarization in DCs we analyzed the intracellular localization of cytokines in DCs during synapse. Abundant stores of intracellular IL-12 are present in DCs 6hr after TLR engagement a time point that corresponds to optimal T cell conjugation capacity. Intracellular stores are mainly associated to the Golgi that is positioned around the MTOC. Upon synapse formation intracellular cytokines are repositioned at the region of interaction with T cells, in close proximity to the T cell membrane. Two lines of evidence suggest that IL-12 are secreted in a polarized fashion at the IS with naïve T cells. First, the volume of the cytokine containing vescicles inversely correlates with the distance from the synapse region. Second, we detect a microtubule dependent burst in secretion of IL-12, as early as 10' after addition of antigen specific T cells in supernatants of DC-T co-cultures. In summary this study illustrates a new mechanism that sustain the ability of DCs to efficienlty prime T cells by repolarization towards the interacting T cell to enhance local delivery of T cell priming mediators.

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HIV-1 MATRIX PROTEIN P17 INDUCES HUMAN PLASMACYTOID DENDRITIC CELLS TO ACQUIRE A MIGRATORY IMMATURE CELL PHENOTYPE

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Numerical and functional defects in plasmacytoid dendritic cells (pDCs) are important hallmark of progressive HIV-1 infection yet its etiology remains obscure. HIV-1 p17 matrix protein (p17), modulates a variety of cellular responses and its biological activity depends on the expression of p17 receptors (p17Rs) on the surface of target cells. In this study, we show that peripheral blood pDCs express p17Rs on their surface and that freshly isolated pDCs are sensitive to p17 stimulation. Upon p17 treatment, pDCs undergo phenotypic differentiation with up-regulation of CCR7. A chemotaxis assay reveals that p17-treated pDCs migrate in response to CCL19, suggesting that these cells may acquire the ability to migrate to secondary lymphoid organs. In contrast, p17 does not induce release of type I IFN nor does it enhance pDC expression of CD80, CD86, CD83 or MHC class II. Microarray gene expression analysis indicated that p17-stimulated pDCs down-regulate the expression of molecules whose functions are crucial for efficient protein synthesis, protection from apoptosis and cell proliferation induction. Based on these results, we propose a model where p17 induces immature circulating pDCs to home in LNs, devoided of their ability to serve as a link between innate and adaptative immune systems.

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CHEMICAL-PHYSICAL FEATURES OF INFLAMED MICROENVIRONMENT MODULATE IL-1ß PROCESSING AND SECRETION

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The role of resident or infiltrating cells and of soluble mediators in the innate immune response to inflammatory noxia have been widely investigated in the last years. Conversely, less attention has been paid on the modifications of chemical-physical parameters such as pH, O2 tension, redox, occurring in inflamed tissues and on their influence on the outcome of the inflammatory response. Here, we show experiments investigating the effects of a microenvironment characterized by different redox conditions, pH and O2 concentrations on the synthesis, processing and secretion of IL-1β induced by several PAMPs in human primary monocytes. IL-1β is a good model for these studies, as this cytokine plays a pivotal role in the inflammatory response. Moreover, unlike other proinflammatory mediators, IL-1β secretion undergo multi-step control mechanisms, at transcriptional, translational and post-translational levels, including processing by caspase-1 following inflammasome assembly, which can all be modulated by extracellular redox, pH and O2 tension.
Our results indicate that extracellular redox does not affect pro-IL-1β expression and intracellular accumulation induced by several PAMPs (including LPS, MDP, Zymosan, flagellin) acting on different TLRs/NOD receptors. However, IL-1β processing and secretion are increased by the presence of reducing agents (DTT or NAC) in the culture medium and decreased by oxidizing agents (H2O2 or As2O3), indicating that a reduced extracellular milieu may potentiate the inflammatory response, whereas oxidizing conditions may result immunesuppressive. We also observed that a low pH (≤ 6) favours processing, whereas at high pH secretion of unprocessed pro-IL-1β is observed. Finally, activation of monocytes in hypoxia results a strong increase in the expression of reducing enzymes such as thioredoxin and in secretion of reduced cysteine, which in turn modulate IL-1β secretion at different extent depending on the PAMP used.

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DO DAMAGE ASSOCIATED MOLECULAR PATTERNS (DAMPs) INDUCE IL-1β SECRETION BY HUMAN MONOCYTES?

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In primary human monocytes, several pathogen associated molecular pattern (PAMP) molecules induce maturation and secretion of IL-1β and IL-18 through a process that involves as a first event the extracellular release of endogenous ATP. Thus, PAMPs acting on different pathogen-sensing receptors (TLRs or NLRs) converge on a common pathway where ATP externalization is the first step in the cascade of events leading to inflammasome activation and IL-1β and IL-18 secretion. Damage associated molecular patterns (DAMP) molecules, released by injured cells in inflamed tissues have been proposed to trigger inflammation through engagement of pathogen-sensing receptors. DAMPs include normal cell constituents and components of the extracellular matrix released by the action of proteases at the site of tissue injury. To compare the inflammatory activities of PAMPs and DAMPs, we studied a number of DAMPs for their ability to trigger synthesis, processing and secretion of IL-1β. Most commercially available DAMP protein preparation indeed induced IL-1β secretion but this effect was prevented by heat-inactivation, indicating that IL-1β secretion was not due to DAMPs but rather to PAMP contamination. In keeping with these results, LPS-free HMGB1 fails to induce IL-1β secretion. However, HMGB1 additioned with low doses of LPS (50pg/ml), incapable to trigger IL-1β secretion per se, acquires the capacity to induce IL-1β synthesis, processing and secretion at levels comparable to those induced by optimal doses of LPS. These results suggest that HMGB1 by itself lacks the minimal requirements to induce IL-1β secretion but may work as a sponge, concentrating minute amounts of PAMPs until their achievement of a stimulatory threshold, or as a presenting molecule, increasing the stimulatory potential of bound PAMPs.

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ARYL HYDROCARBON RECEPTOR (AHR) ACTIVATION PERTURBS MYELOID SUBLINEAGE DIFFERENTIATION BY INHIBITING PU.1 UPREGULATION

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The ligand-induced transcription factor AhR represents a promising therapeutic target in allergy and autoimmunity. Certain AhR ligands induce regulatory T cells and inhibit autoimmunity. In contrast the new AhR ligand VAF347 effectively suppressed allergic lung disease likely via suppressed dendritic cell (DC) function. How AhR ligands alter DC function remained undefined. As a model for epithelial DCs we studied AhR effects on human in vitro generated Langerhans cells (LCs). Furthermore we compared these cells to other myeloid cell types. AhR is highly regulated during sub-lineage differentiation of myeloid progenitor cells. LCs expressed highest AhR levels followed by monocytes; conversely neutrophils lacked detectable AhR expression. Various AhR ligands including VAF347 arrested the differentiaton of monocytes and LCs at an early monopoietic precursor cell stage, whereas progenitor cell expansion or granulopoiesis remained unimpaired. AhR was co-regulated with the transcription factor PU.1 during myeloid subset differentiation. AhR activation inhibited PU.1 induction during initial monocytic differentiation, and ectopic PU.1 restored monocyte and LC generation in the presence of VAF347. AhR ligands failed to interfere with cytokine receptor signals (TGF-b1 or TNFa) essential for LC generation, and failed to impair maturation of immature LCs.
These data suggest that AhR-mediated suppression of epithelial DCs is mediated by impairment of monocytic precursor generation from myeloid progenitors and this effect is mediated by impaired PU.1 induction. We propose that AhR-mediated targeting of monocytic precursors is a promising new mechanism of therapeutic immunosuppression.

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DC-ATLAS: a curated database of the pathways of the dendritic cell.

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What is a pathway is still an open question. Pathways are more than a gene list pathways describing both the type of interaction, direction and extent of interaction of elements within a cell and between cells, in time.
The T cells stimulatory potential of DCs depends on the duration and combination of stimulatory signals. DC activation should be described trough the pathways integrating multiple stimuli over a defined temporal window, time and place should be taken in consideration when describing the sequence of events.
Here we describe DC-ATLAS an initiative, developed in the context of "Dendritic Cells & Novel Immunotherapies" (DC-THERA) European N.O.E., to integrate the signalling modules of the dendritic cell curating specifically: A-the pathways leading from, immature dendritic cells to mature dendritic cells. B- pathways depending on interaction between different cell types i.e. DCs and T cells interaction, leading to Th17 differentiation, and apoptosis.
DCs differ in type, origin and their biology is significantly different in Homo sapiens and Mus musculus. Given the complexity of the problem we have selected a group of curators with the aim propose to describe separately: 1-the section of the pathway sensing the stimulus and transmitting the stimulus to general signal transduction pathway. 2-the principal signal transduction modules used by the DC. 3- the outcome: interleukin production, apoptosis, migration, differentiation. The first action we have undertaken is defining a data model to describe the pathways of the dendritic cell. The DC pathways will be described in terms of proteins participating to the intracellular cascade of events. These proteins will be linked to an expected readout, i.e. IL production, maturation, apoptosis. The signal will be passed from a protein the another as protein phosporilation or modification, complex formation and will often result in changes in gene expression. The abstraction of these concept will be a network where the nodes are the proteins and the lines are the interactions.
As a result these pathways will be amenable to reconstruct the hierarchy of events. Annotating these pathways in the context of the integration of immune response with metabolic alterations of the cell will be also important to uncover the "tempo" of the response.
The pathways will be annotated in the pathway database of the Eu-Gene bioinformatics environment(Cavalieri 2007) and used to assess statistical enrichment of specific cellular modules of microarray gene expression and proteomics datasets obtained on dendritic cells exposed to a plethora of maturation stimuli. The ultimate aim of this project will be to use microarrays to develop markers at the pathway level to guide immunotherapy.
Cavalieri, D., Castagnini, C., Toti, S., Maciag, K.,Kelder, T., Gambineri, L., Angioli S., Dolara, P. Eu.Gene Analyzer a tool for integrating gene expression data with pathway databases. 2007 Jun 28; Bioinformatics.

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THE ROLE OF NITRIC OXIDE AND ARGINASE FOR THE NO-SYNTHASE-MEDIATED KILLING OF INTRACELLULAR LEISHMANIA PARASITES IN MACROPHAGES

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Control of the intracellular parasite Leishmania in macrophages requires inducible nitric oxide synthase (iNOS), which metabolizes L-arginine into citrulline and nitric oxide (NO). iNOS can be antagonized by arginase (Arg) which converts arginine into urea and ornithine, a known precursor of polyamines that are essential for eukaryotic cell growth. Arg is expressed by the parasites themselves, but also by host cells such as macrophages following activation by interleukin (IL)-4, IL-10, IL-13 or TGF-beta. Previously it was postulated that iNOS-dependent killing of Leishmania is not mediated by NO itself, but by N-hydroxy-L-arginine (LOHA), the intermediate product of the iNOS-reaction, which inhibits Arg and thereby the parasite growth.

Here, we present a panel of data supporting the idea of direct killing of the intracellular parasites by the toxic effects of NO. (a) Addition of a NO-scavenger to L. major-infected macrophages stimulated by interferon (IFN)-gamma and tumor necrosis factor (TNF) led to a significant increase of parasite proliferation. (b) Transwell and coculture experiments with infected iNOS-/- macrophages and IFN-gamma/LPS-stimulated wildtype macrophages revealed that the destruction of Leishmania in the iNOS-/- cells required the close proximity of iNOS+/+ macrophages, indicating that unstable and reactive NO mediated the kill. (c) LOHA alone failed to cause killing of Leishmania in macrophages and ornithine was unable to reverse the antileishmanial activity of IFN-gamma/TNF-stimulated macrophages. (d) There was no difference in the parasite survival between infected Arg I-/- and wildtype macrophages. (e) Intracellular L. major were killed by NO-donors specifically targeted to the phagosomal compartment by endocytosis. Together, these data led us to conclude that iNOS-dependent killing of intracellular Leishmania parasites depends on direct toxic effects of NO rather than on indirect effects of reduced parasite growth due to diminished arginase activity.

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INTRAVENOUS IMMUNOGLOBULINS DIMINISH THE NUMBER OF PROINFLAMMATORY CD14+CD16+ MONOCYTES IN PERIPHERAL BLOOD OF CVID PATIENTS.

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Human peripheral blood monocytes can be subdivided into CD14+CD16+ cells (”proinflammatory monocytes”) and CD14++ CD16- cells (“classical monocytes”). The CD14+CD16+ monocytes form about 10% of all monocytes and they produce large amounts of the pro-inflammatory cytokines - TNF and IL-12 but little or no anti-inflammatory IL-10. We have used a 4-color flow cytometry and single platform assay to determine the absolute numbers of monocytes in patients with immunodeficiencies. This included adult (n=13) and pediatric (n=38) patients with common variable immunodeficiency (CVID), transient hypogammaglobulinemia of infancy (THI, n=22) and selective IgA deficiency (SIgAD, n=41), in comparison to healthy, age matched control subjects. Monocyte numbers did not show any abnormalities in these humoral immunodeficiences.

In adults with CVID we did, however, detect a decrease of CD14+CD16+ monocytes by about 50% at 4 hrs following infusion of intravenous immunoglobulins (IVIG at 0,4 g/kg body weight). At the same time levels of TNF and IL-12p70 protein in the supernatants of phorbolester stimulated peripheral blood mononuclear cells decreased by about 40% and 70%, respectively, while IL-10 levels were unchanged. We postulate that this action on monocytes may explain the anti-inflammatory effect of IVIG therapy.

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PROLIFERATION AND MIGRATION OF MACROPHAGES IS ENHANCED BY SPHINGOSINE-1-PHOSPHATE (S1P) AND pFTY720

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Objective: Sphingosine-1-phosphate (S1P) plays an important role in a number of biological processes relevant to vascular biology including immunity, angiogenesis and regulation of endothelial and SMC biology. Recently, we described a potent anti-atherogenic effect of the S1P-analogue FTY720 in ApoE-deficient mice that was associated with a reduction of lesional macrophage content. However, the direct effect of S1P on macrophage function is less known. We therefore investigated the effect of S1P and FTY720 on proliferation and migration of mouse macrophages (RAW) and peritoneal macrophages of C57BL/6 and S1P3 deficient mice. Methods and Results: S1P (100 nM) stimulated proliferation of RAW macrophages by 18 % and 64 % after 48 and 72 hours as measured by determination of cell numbers. pFTY720 (the biologically active form of FTY720; 100 nM) also increased proliferation (58 % after 72 h). In addition, S1P and pFTY720 potently stimulated migration of RAW macrophages as measured in Boyden chamber experiments (269 % and 136 % as compared to controls, respectively). Thioglycolate-elicited peritoneal macrophages as well as bone marrow-derived macrophages expressed all three S1P receptors with S1P1 as the most abundant receptor followed by S1P2 and S1P3 as determined by Real-time PCR. In the peritonitis model in vivo, we observed a 50% reduction of peritoneal macrophage numbers in S1P3-deficient mice after 4 days compared to controls. We also measured a substantial increase of S1P in the inflamed peritoneal cavity by HPLC. In vitro, S1P stimulated migration of wild type peritoneal macrophages but had no migratory effect in macrophages from S1P3 deficient mice. We also observed differences in the expression of several cytokines such as MCP-1, TNF-a and IFN-g between macrophages of wild type and S1P3 deficient mice as determined by Real-time PCR. These results implicate that the S1P3 receptor is essential for recruitment of macrophages into the peritoneal cavity during inflammation.

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DIFFERENT FORMS OF Saccharomyces Cerevisiae ELICIT SPECIFIC RESPONSES IN DENDRITIC CELLS

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How our immune system discriminates between pathogenic and non pathogenic microorganisms is still an open question. We propose to use different vegetative and non vegetative forms of the non pathogenic Saccharomyces cerevisae yeast to investigate the rules of the recognition game. Phagocytosis-mediated antigen presentation together with TLR-dependent gene expression of inflammatory cytokines and co-stimulatory molecules, instruct development of antigen-specific acquired immunity. Monocyte-derived dendritic cell (MoDC) recognize S. cerevisiae yeast trough different TLRs such as TLR4 and TLR2. TLR-2 is essential in the recognition of fungal-derived components in collaboration with dectin-1, a lectin family receptor for the fungal cell wall component ß-glucan. After recognition of cell wall components, MoDC phagocytise yeast cells and nucleic acids, in particular ssRNA, are recognized by intracellular TLRs such as TLR7, TLR8 and TLR9 eliciting an additional robust pro-inflammatory response with cytokine production. In order to deeper insight the mechanisms of DC-S. cerevisiae interaction, we evaluated the role of RNA and different cell wall in different conditions. The different composition of cell wall in different growth conditions may influence the immune recognition of this yeast. We evaluated the ability of vegetative and senescent cells of S. cerevisiae to induce MoDC cytokine responses. By measuring cytokine production we observed that cells in different senescence states elicit peculiar immunological response. To reconstruct the signalling networks responsible for differences in the response we have performed transcriptional analysis of hMoDC and used Eu.Gene (Cavalieri et. al, 2007) to reconstruct pathways and regulatory programs.

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EXPRESSION OF CHOLINERGIC COMPONENTS IN MONOCYTIC THP-1 CELLS IN INFLAMMATION

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Acetylcholine (ACh) is a well-known neurotransmitter and vasodilator. Recently, it has also been ascribed an anti-inflammatory role in the context of the cholinergic anti-inflammatory pathway. Here, ACh binds and activates the nicotinic receptor nAChR-a7 in inflammatory cells, such as monocytes and macrophages, and leads to decreased production of pro-inflammatory cytokines. In cholinergic systems, ACh content and activity is regulated at its synthesis and degradation steps. Taken this, we have hypothesized that if expressed by monocytes/macrophages, cholinergic components ensuring these steps could play a role in modulating the inflammatory response. To address this, we have here investigated the expression of those cholinergic components in THP-1 cells (a human acute leukemic monocytic cell line) in the absence and presence of a known inflammatory stimulus, lipopolysaccharide, LPS. By RT-PCR and according to published data, we confirmed that THP-1 cells express the nAChR-a7 receptor and demonstrated that LPS does not modify nAChR-a7 expression. We further established that these cells do not express choline acetyltransferase (ChAT), the enzyme responsible for ACh synthesis. Moreover, ChAT expression is not induced upon incubation with LPS. These results indicate that THP-1 cells should not able to produce and secrete ACh. On the other hand, we showed here that THP-1 cells express the enzyme responsible for ACh degradation, acetycholinesterase (AChE). Importantly, LPS induction was shown to up-regulate AChE mRNA expression. Nevertheless, Western-blotting studies further revealed that AChE protein content remains unaltered under these conditions. These results suggest the existence of post-transcriptional mechanisms that silence AChE expression in monocytes during the inflammatory response.

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FUNCTIONAL ANALYSIS OF THE IREM-2 RECEPTOR (CD300e) IN HUMAN MONOCYTES

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IREM-2 (CD300e) was originally reported to be expressed by mature monocytes and peripheral blood myeloid dendritic cells. The receptor was associated to the DAP-12 adaptor in co-transfected cells, and induced NFAT transcriptional activity in a reporter assay upon engagement by a specific mAb (Aguilar et al. 2004. J. Immunol. 173: 6703-6711). We have investigated the functional effects of CD300e ligation in various responses of human monocytes. Upon engagement by an agonistic mAb, CD300e triggered an intracellular calcium mobilization and the release of reactive oxygen intermediates in freshly isolated cells. Stimulation via CD300e triggered the production of pro-inflammatory chemokines and cytokines) and up-regulated the expression of cell surface)(i.e. IL-8/CXCL8 and TNFα co-stimulatory molecules (i.e. CD83 and CD86). CD300e ligation promoted monocyte survival in culture, increasing their phagocytic activity. Overall, our data demonstrate that CD300e functions as an activating receptor in human monocytes regulating the innate immune response.

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CHARACTERISATION OF DENDRITIC CELL FUNCTIONAL DEFECTS IN WISKOTT-ALDRICH SYNDROME

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Wiskott-Aldrich Syndrome (WAS) is a rare X-linked primary immunodeficiency caused by defective expression of the WAS protein (WASP) in haematopoietic cells. Several immune functions in T and B lymphocytes, platelets, dendritic cells (DCs), macrophages, natural killer (NK) cells and neutrophils of WAS patients are altered. This results in many clinical manifestations that include recurrent infections, eczema, thrombocytopenia, and increased risk of autoimmune disorders and lymphomas. Increasing evidences show the contribution of DC defects in WAS pathogenesis. Reduction of WASP expression impairs cytoskeleton organization and inhibits podosome formation in DCs and dramatically reduces their functionality. In the present work, we have focused our attention on two aspects of WAS pathogenesis in which DCs are involved: the defective pathogen clearance and autoimmune manifestations. We show preliminary results about DC homeostasis and functionality in was murine model. Analysis of DC subset distribution (lymphoid DCs and myeloid DCs) in spleen, lymph nodes (LNs) and thymus of was-/- mouse does not show any gross abnormalities. In spite of normal DC distribution, some DC functions show to be defective. In particular, our preliminary findings confirm previous observation on defective DC ability to migrate to draining LNs. As a consequence of an abnormal cytoarchitecture, we hypothesize that also DC capability to contact and uptake antigens may be impaired. Our analysis shows that was-/- DCs are less efficient to uptake latex beads than wt DCs. As it has been shown that cytoskeleton defects can impair the formation of immunological synapse between DCs and T cells, we tested DC ability to prime T cells in vitro. We found a reduced proliferation of T cells cultured in the presence of was-/- DCs. These data are consistent with the hypothesis that in the lack of WASP, duration of DC-T cell contact may be altered as well as the recruitment of costimulatory molecules in the synapse. Since we and others have previously demonstrated the efficacy of gene therapy approach to correct T cell defects, we have evaluated DC functionality in genetically corrected was-/- mice. Interestingly, we found that DCs obtained from gene therapy treated was-/- mice show amelioration in the capacity to migrate to draining LNs. Finally, to further investigate how DCs contribute to autoimmune manifestation in WAS, we have analysed thymic morphology and clonal selection in was-/- mice. Analysis of positive selection and thymocyte differentiation does not reveal any alteration. Nevertheless, morphological analysis of was-/- thymi shows thymic hypoplasia and less clear definition of the cortico-medullary junction, where immature DCs normally reside. All these findings provide a strong rationale to further investigate DC defects to better define their involvement in pathogenesis of Wiskott-Aldrich Syndrome.

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THE METHODOLOGICAL APPROACH FOR THE GENERATION OF HUMAN DENDRITIC CELLS FROM MONOCYTES AFFECTS THE MATURATION STATE OF RESULTANT DENDRITIC CELLS

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Dendritic cells (DCs), the most important antigen-presenting cells, exist in two maturation state, immature DCs, that have the function of capturing, processing and presenting antigens, and mature DCs, that develop immunomodulatory capacities, inducing the immune response.
Usually, DCs can be in vitro generated culturing CD14+ monocytes with growth factors. To obtain high purified CD14+ cells, immunomagnetic selection methods are usually employed. Our study focuses on two of them, the former developed by Miltenyi Biotec (MACS® Separation), the latter by StemCell Technologies Inc. (EasySep® Selection). Valuing the differences between both systems concerning the components and the principals of the methodological approaches, we would make a comparison in order to establish which provides a better cell yield and purity, available for functional and phenotypic assays. Therefore, we generate monocytes from peripheral blood mononuclear cells through the two above named immunomagnetic selection methods and we properly differentiate them in dendritic cells in a culture medium with GM-CSF and IL-4 for 6 days. DC maturation is induced by lipopolysaccharide (LPS) addition on day 6. Phenotypical evaluation (for costimulatory molecules as CD40 and CD80, for HLA-DR antigen and, above all, for the maturation marker CD83) and functional analyses (endocytic capacity and interleukin (IL)-1, IL-6, IL-10, IL-12, tumor necrosis factor-α (TNF-α), transforming grow factor-β (TGF-β), vascular endothelial growth factor (VEGF) and CD40-ligand (CD40-L) cytokines production) are performed by cytometer on day 6 (to analyse immature DCs) and on day 7 (to analyse mature DCs). Our results demonstrate that both systems permit to obtain monocytes with high purity, without affecting DC generation. Morphological analysis, phenotypical evaluation and functional investigation show that MACS®-DCs have the expected DC features on days 6 and 7, while EasySep®-DCs present a partially mature state since day 6. We provide evidences that the partial maturation is probably due to the dextran-coat of EasySep®-beads. Indeed DCs could capture the beads through an endocytic pathway mediated by mannose receptor, that contains multiple carbohydrate-bindings domains for complexes that expose mannose residues. Our thesis is also supported by the presence of yellow vacuoles inside the cytoplasm of EasySep®-generated monocytes and DCs: they could represent the internalized beads. Conclusively, even if EasySep® is a useful method concerning to costs, rapidity and purity, we recommend to use MACS® positive selection system, whereas dendritic cells at totally immature state (fundamental for the functional studies) are requested.

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DICER IS REQUIRED FOR INVARIANT NKT CELL DEVELOPMENT

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Invariant Natural killer T (iNKT) cells are a separate lineage of innate-like T lymphocytes that express a conserved invariant TCR made of the invariant Va14-Ja18 rearrangements paired with Vb8.2, -7 or -2 in mice. iNKT cells recognize both self and exogenous lipids (like α-Galactosyl-Ceramide) presented by CD1d. iNKT cells undergo a distinct pathway of thymic development and effector differentiation, that depend on the activity of signal transduction molecules or transcription factors that, by contrast, play minor or no role for MHC restricted T cells. This prompted us to hypothesize that the peculiar developmental pathway of iNKT cells could depend also on a lineage-specific control of gene regulation by microRNAs (miRNAs). miRNAs are highly conserved small RNA molecules that post-transcriptionally regulate gene expression binding to the 3'-untranslated regions (3'-UTR) of specific target mRNAs, leading to translation inhibition or mRNA degradation. We have therefore investigated the iNKT cell development in mice depleted of miRNAs by eliminating Dicer, the RNAse III enzyme that generates functional miRNAs, on cortical immature thymocytes from the double positive (CD4+ CD8+) stage.
The lack of Dicer causes a dramatic decrease (more than 100 fold) in thymic and peripheral iNKT cells in comparison to Dicer-expressing animals. By contrast, the lack of Dicer did not affect thymic MHC-restricted T cells and caused a five-fold reduction in their peripheral number. We excluded an altered expression of CD1d and Slam family members on DP thymocytes, the molecules and the cell type crucial for iNKT cell selection, expansion and maturation. DP thymocytes from Dicer KO mice presented normally α-GalactosylCeramide to iNKT cells hybridoma, whereas iNKT cells from Dicer KO mice did not respond to α-GalactosylCeramide in vitro. Finally, Reaggregate Thymic Organ Cultures confirmed that lack of Dicer impairs iNKT cell development in a cell-autonomous manner. Moreover, preliminary results suggested that NKT cells development is arrested at an early stage in the thymus.

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Molecular signature of iNKT cells undergoing altered effector differentation

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Invariant NKT (iNKT) cells are a subset of autoreactive T lymphocytes with innate effector functions that expess a semi-invariant TCR specific for lipids, such as aGalactosylCermide (aGalCer), presented by CD1d. Unlike conventional MHC-restricted T cells, iNKT cells are selected by DP thymocytes and not by thymic epithelial cells, and undergo an innate like differentiation program immediately after thymic selection. Cortical thymocytes and DCs express CD1d and are involved in the positive and negative selection of iNKT cell. We have shown in a transgenic mouse model that targeting CD1d expression on thymocytes is sufficient for thymic development and differentiation of iNKT cells. In this GalCerαmodel, however, iNKT cells are markedly hyper responsive in vitro to dependent activation in comparison to wt iNKT cells, suggesting that they underwent an altered effector differentiation upon incomplete CD1d-dependent cellular interactions. To investigate the molecular bases for the altered effector developement, we performed a comparative gene expression analysis of tg and wt iNKT cells ex vivo or after aGalCer-dependent activation in vitro. The analysis identifies a molecular signature that characterizes the tg iNKT cells ex vivo and accounts for their Ag-hyper responsiveness. In particular, tg iNKT cells express significantly lower amount of SHP-1, a tyrosine phosphatase involved in the negative regulation of TCR signaling, than wt iNKT cells. These results suggest that the correct effector development of iNKT cells requires the interaction with CD1d expressed on cells other then thymocytes to fine tune the expression of molecules critical for their reactivity. We are trying to normalise the molecular signature and reactivity of tg iNKT cells by reintroducing, through thymic transplants or mixed bone marrow radiation chimeras, the interaction with DC cells expressing CD1d.

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FUNCTIONAL ROLE OF ARHGAP 15, A NEW RACGAP PI3K-DEPENDENT

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The Rho GTPases such as Cdc42, Rac1 and RhoA regulate diverse biological processes including actin cytoskeletal dynamics, cell adhesion, cell polarity and migration. Through their intrinsic GTPase activity, small GTPases switch off by converting this GTP to GDP. Under cellular conditions the intrinsic rate of exchange of bound nucleotide is low and small GTPases are inhibited by factors enhancing their GTPase activity (GAPs). ArhGAP15 is a recently cloned RacGAP with a pleckstrin homology (PH) domain at C-terminal and a RhoGAP domain at N-terminal. The GAP domain of ArhGAP15 showed specificity towards Rac1 in vitro. The PH domain is required for ArhGAP15 to localize on plasma membrane. Coexpression analysis with bioinformatic tools revealed that ArhGAP15 coexists in leukocytes with PI3Kgamma, the unique class IB of PI3K that is specifically activated by G protein-coupled receptor and that has a central role in leukocyte migration. Here we show that ArhGAP15 appears localized in the cytoplasm in resting conditions and is translocated to the plasma membrane upon C5a stimulation in bone marrow-derived macrophages. Treatment with the PI3K inhibitor LY294002 significantly reduced ArhGAP15 membrane localization in stimulated cells, indicating that is membrane localization and activation is PI3K-dependent. These results indicate ArhGAP15 as a possible PI3K effector. To address the in vivo relevance of this protein, the current study examined the effect of the knock-down and over-expression of ArhGAP15 in murine macrophages. The loss of ArhGAP15 induces multiple alterations of macrophage cellular behavior known to be under the control of Rac. Macrophages lacking ArhGAP15 exhibited an atypical, elongated morphology. Moreover downregulation of ArhGAP15 induces an increased motility whereas the over-expression causes a defective migration. These results identify ArhGAP15 as a GAPs that specifically negatively regulate Rac function in vivo in primary macrophages.

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DENDRITIC CELL MODELS OF AUTOIMMUNE DISEASE FOR SCREENING OF ANTI-INFLAMMATORY DRUGS AND PROBIOTICS

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Dendritic cells are attractive target cells for treatment of inflammatory diseases within both autoimmunity and allergy. Monocytes are believed to migrate into the tissue and differentiate into immature DCs, which are attracted to sites of inflammation due to presence of pro-inflammatory cytokines and chemokines. During this process immatureDCs become inflammatory DCs that migrate to lymph nodes and activate the adaptive immune system. The lifespan of DCs is therefore relatively short at sites of inflammation, and the inflammatory DCs are believed to promote the inflammatory process. This makes DCs an attractive therapeutic target cell for treatment of inflammatory diseases. We have designed a human DC-based in vitro screening model for identification of anti-inflammatory compounds and microorganisms like probiotics. To mimick the situation in inflammatory disease like Crohns disease we have designed cocktails consisting of pro-inflammatory cytokines and TLR-agonists, known to be involved in Crohns disease. Several cocktails were identified, which are able to induce secretion of pro-inflammatory mediators from the DCs like IL12p70, IL23, TNF-alfa and prostaglandin E2. The cocktails also induced expression of DC maturation markers and showed an ability to stimulate a mixed leukocyte reaction after addition of CD4+ T cells. The model was validated using known clinically used anti-inflammatory drugs like dexamethasone and COX-inhibitors, which were able to suppress the maturation of the DCs into the inflammatory DC phenotype. Several probiotic strains were shown to potently suppress the inflammatory DC development, and would hence be attractive candidates for clinical testing in autoimmune diseases like Inflammatory Bowel Disease.

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AXL REGULATES SURVIVAL AND MIGRATION OF HUMAN DENDRITIC CELLS

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Axl is the prototype of a family of transmembrane tyrosine kinase receptors (TAM), which includes Tyro3, and Mer. Gas6, the product of growth arrest–specific gene, binds all three receptors although a greatest affinity for Axl has been reported. Novel roles for the Gas6-Axl system in innate immunity are becoming increasingly apparent. Here we show that human monocyte-derived dendritic cells (DC) generated in the presence of GM-CSF and IFN-alpha (IFN/DC) express Axl on their surface, whereas Axl expression could not be detected in DC generated with GM-CSF and IL-4 (IL-4/DC). IFN-alpha treatment of IL-4/DC results in Axl upregulation to levels comparable to the ones of IFN/DC. IFN-alpha specifically induced Axl, but did not affect Tyro3 and Mer expression. Maturation stimuli (LPS, Poly:IC, Imiquimod) significantly downregulated Axl expression by IFN/DC through increased proteolytic cleavage. The reduction of surface expression was associated with elevated levels of the soluble form of Axl in the supernatants of mature IFN/DC. Moreover GM6001, a general inhibitor of MMP- and ADAM-family proteases, restored the expression of Axl on the cell surface and diminished its concentration in the supernatants. Evaluating the effect of Gas6 on Axl-expressing DC, we found that Axl expressing DC migrate in response to Gas6 and that Gas6 decreased serum-deprivation-induced apoptosis in IFN/DC. In during human DC summary, our study reveals that Axl is upregulated by IFN-alpha differention/activation. Furthermore, Gas6 regulates DC migration and guarantees for DC homeostasis. Dysregulation in Axl expression or Gas6 availability could therefore affect the functionality of DC in autoimmunity and anti-viral immune responses characterized by high levels of IFN-alpha.

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ACTIVIN A INDUCES LANGERHANS CELL DIFFERENTIATION

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Langerhans cells (LC) represent a well characterized subset of dendritic cells located in the epidermis of skin and mucosae. In vivo, they originate from resident and blood-borne precursors in the presence of keratinocyte-derived TGFbeta. In vitro, LC can be generated from monocytes in the presence of GM-CSF, IL-4 and TGFbeta. However, the signals that induce LC during an inflammatory reaction are not fully investigated. We report that Activin A, a TGFbeta family member induced by pro-inflammatory cytokines and involved in skin morphogenesis and wound healing, induces the differentiation of human monocytes into LC in the absence of TGFbeta. Activin A-induced LC are Langerin+, Birbeck granules+, E-cadherin+, CLA+ and CCR6+ and possess typical APC functions. In human skin explants, intradermal injection of Activin A increased the number of CD1a+ and Langerin+ cells in both the epidermis and dermis by promoting the differentiation of resident precursors. High levels of Activin A were present in the upper epidermal layers and in the dermis of Lichen Planus biopsies in association with a marked infiltration of CD1a+ and Langerin+ cells. These results support the existence of a novel LC differentiation pathway, specifically dependent on Activin A, which might be relevant in pathological conditions.

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MUTUAL CYTOTOXIC REACTIONS BETWEEN DECIDUAL NK AND DENDRITIC CELLS

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Problem: The aim of this study was to investigate possible mutual cytotoxic reactions and mechanisms employed by decidual DC and CD56+ cells. Design and methods: Decidual mononuclear cells (DMC), obtained from early human pregnancy decidua after enzymatic digestion and gradient centrifugation were cultured overnight at 37oC. Non adherent DMC fraction was used for positive magnetic separation of CD56+ cells and enrichment of CD83+ cells from «CD56 negative» fraction. Decidual CD1a+ cells were separated from decidual adherent cells. The expression of cytotoxicity receptors were performed in CD56+ cells and CD1a+CD56- cells by flow cytometry. Perforin, granulysin, Fas ligand (FasL), tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and serine asterases were investigated by RT-PCR, flow cytometry and/or confocal microscopy in decidual NK or DC, respectively. Cytotoxicity of NK cells against DC and vice versa were measured by 2-hours PKH-26 cytotoxicity assay. In some assays monoclonal antibodies (mAb) toward cytolytic molecules, NK cell receptors or MHC class I chain related –A/B (MIC A/B) antigen were used, respectively. Results: CD1a+ cells from freshly isolated DMC suspensions express pro-inflammatory cytokines /IL-15 and IL-18 (30-45%), IFN-gamma (~15%) and TNF-alpha (~6%)/, cytolytic mediators (perforin, granulysin, FasL and TAIL) and CD94, NKG2D in higher percentage. These cells were able to kill cognate decidual NK cells from 5-15% (effector:target ratios 6:1 - 25:1) by perforin mechanism. Decidual CD56+ cells, riched in cytolytic granules, kill significantly less CD1a+ cells in comparison to CD83+ cells, although perforin caused both lyses. Additional effects of anti-FasL, anti-TRAIL and anti-granulysin mAb were observed neither in DC nor in CD56+ cells killing. NKG2D is expressed on positively selected CD56+ cells, but decidual DC are devoid of MIC A/B, that way anti-NKG2D and anti-MIC A/B mAb did not affect killing of DC. Anti-NKp46 mAb completely abrogated NK cell killing of CD1a+ cells, but not those of CD83+ cells, thus the effects of anti-NKp44 oe anti-NKp30 was not observed. Conclusions: Decidual CD1a+ cells are able to produce pro-inflammatory cytokines, cytolytic mediators and show selective cytotoxicity against cognate NK cells. CD1a+ cells are somehow protected more than CD83+ cells from NK cell mediated lysis, although CD1a+ DC are able to bind for NKp46 receptor on decidual NK cells providing that way activating signal in NK cells for their own lyses. Perforin seems to be main cytolytic mediator imployed in regulation of leukocyte subpopulations at the maternal-fetal interface. Acknowledgement: The experiments were financed by EMBIC project European FP6, NoE, LSHM-CT-2004-512040 and Croatian Ministry of Science, Education and Sports (Grants No. 0620402-0376 and No. 0377).

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TUMOR ASSOCIATED GLYCOPROTEIN-72 AND MUCIN I SHOW DISTINCT IMPACT ON DECIDUAL DENDRITIC CELLS FUNCTIONS

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Problem: Possible binding and internalization of endogenous ligands Tumor Associated Glycoprotein-72 (TAG-72) and mucin I (MUC I) by decidual CD1a+ dendritic cells (DC), as well as their influence on DC phenotype, cyto/chemokine production were investigated.

Material and methods: Decidual mononuclear cells (DMC) were obtained from the first trimester normal human pregnancy decidua by enzymatic digestion (Collagenase IV) and gradient density centrifugation (Lymphoprep). DMC immediately after isolation were untreated or pretreated with TAG-72, MUC I or mannan for 30 min on ice and then CD209 and CD206 were labeled using antibody directed toward their carbohydrate recognition domains or FITC dextran pinoendocytosis was measured by flow cytometry. Intracellular IFN-gamma, IL-4, IL-10, IL-15 and IL18 cytokines were detected in CD1a+ dendritic cells (DC) from DMC suspensions recovered after 18-hours culture in the medium only or in the presence of TAG-72 or MUC I, respectively. The chemokines CXCL10 and CCL17 were analyzed in TAG-72 or MUC I treated or untreated enriched decidual CD1a+ supernatants by enzyme linked immunosorbent assay (ELISA). Mann-Whitney U test was used to determine statistical significance between two groups of interest. Results: Both, TAG-72 and MUC I, as well as mannan (positive control) significantly reduced anti-CD206 and anti-CD209 mAb binding and FITC dextran endocytosis on a dose dependent manner in early pregnancy decidual CD1a+ cells. TAG-72 decreased CD83 surface expression on CD1a+ cells, whereas MUC I caused up-regulation of IL-1R type II and D6 pro-inflammatory decoy receptors. The addition of TAG-72 in the suspension of DMC minced substantially the percentage expressing CD1a+ DC and mean fluorescence intensity (MFI) forγof IL-15 and IFN- IL-15 and IL-18 in CD1a+ cells, whereas such effects were not noticed in MUC I treated cells. Neither TAG-72 nor MUC I affected IL-4 and IL-10 expression in DC. CXCL10 secretion (approximately 40 ng/ml) was four times higher than CCL17 secretion of enriched decidual CD1a+ cells cultured in the medium only and it became balanced in the presence of TAG-72. Conclusions: Decidual CD1a+ cells were able to bind and internalize both endogenous ligands tested by carbohydrate recognition domains of CD206 and CD209 receptors, but their effects differed. TAG-72 efficiently down-regulate Th1 oriented cytokine/chemokine production, whereas MUC I up-regulated pro-inflammatory decoy receptor expression. Acknowledgement: The experiments were financed by EMBIC project European FP6, NoE, LSHM-CT-2004-512040 and Croatian Ministry of Science, Education and Sports (Grants No. 0620402-0376 and No. 0377).

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IL-10 AND NF-kB P50 LIMIT DIFFERENTIATION OF MONOCYTES TO PATHOGENIC TNF/INOS-PRODUCING DENDRITIC CELLS DURING PARASITIC INFECTION

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The development of classically activated macrophages (M1) is a prerequisite for effective elimination of parasitic, bacterial or viral pathogens. However, if the activation of M1 persists, pathogenic damage to cells and tissues ensues. Therefore, we aim to identify the mechanisms by which M1 contribute to tissue damage in order to dampen their pathogenicity and increase the resistance of the host to parasitic diseases. Pathogenic features of infection with the extracellular African trypanosomes include anemia and liver injury that result in loss of parasite control capacity and death of the host. We established that monocyte-derived TIP-DCs represented a prominent M1 subpopulation that contributed actively to the pathogenicity of African trypanosomiasis by being the major source of TNF and NO. The conversion of monocytes to TIP-DCs was a two-step process, involving an IFN-g/MyD88 signaling independent differentiation step from monocytes to immature inflammatory DCs, followed by an IFN-g/MyD88 signaling dependent maturation step to TIP-DCs. CCR2 promoted the egression of monocytes from the bone marrow of infected mice but not their differentiation and maturation into TIP-DCs. As a consequence, CCR2 KO mice infected with trypanosomes experienced reduced pathogenic symptoms. On the other hand, the absence of IL-10 enhanced the differentiation of monocytes to TIP-DCs, resulting in exacerbated pathogenicity and early death of the host. We demonstrated that, following therapeutic liver-specific delivery of the IL-10 gene (using Adeno-Associated Viral vector) in chronically T. brucei infected mice, IL-10 did not impair the recruitment of monocytes in inflamed organs, but efficiently limits their in situ differentiation and maturation in inflamed organs to TIP-DCs, hereby preventing the pathogenicity of the disease without affecting the clearance capacity of the host. Finally, accumulated evidence suggested that, in the absence of the NF-kB member p50, the maturation of inflammatory DCs to TIP-DCs increased, resulting in increased production of the pathogenic molecules TNF and NO and associated tissue injury culminating in early dead of the host. Together our data demonstrate that hosts confronted with a chronic inflammatory situation can be protected from tissue damage by preventing the differentiation/maturation of monocytes to TIP-DCs through IL-10 gene delivery or through NF-kB p50 overexpression, which establishes a novel paradigm for regulating TIP-DC function in vivo.

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CCR2 mediates homeostatic and inflammatory release of Gr1HI monocytes from the bone-marrow, but is dispensable for bladder infiltration in bacterial urinary tract infection

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The C-C motif chemokine receptor 2 (CCR2) is thought to recruit monocytes to sites of infection. Two subpopulations of murine blood monocytes differing in Gr1 (Ly-6C) and CCR2 expression have been described. The exact role of CCR2 in migration of CCR2LO Gr1LO and CCR2HI Gr1HI monocytes into non-lymphoid tissue is controversial. Here, we have addressed this question in a murine model of bacterial urinary tract infection. Only Gr1HI monocytes were recruited into the infected bladder. CCR2-deficiency reduced their frequency in the organs, indicating a requirement of this chemokine receptor. Importantly, CCR2-deficient mice also showed reduced Gr1HI monocyte numbers in the blood, but not in the BM, indicating that CCR2 acted at the step of monocyte release into the circulation. The same was found also in non-infected mice, indicating a further involvement of CCR2 in steady-state BM egress. An additional requirement of CCR2 in monocyte recruitment from the blood into the bladder was excluded by tracking particle-labeled endogenous monocytes and by adoptive transfer of BM-derived monocyte subsets. These findings demonstrate that CCR2 governs homeostatic and infection-triggered release of Gr1HI monocytes from the BM into the blood, but is dispensable for recruitment into a non-lymphoid tissue.

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IMMUNE INFILTRATES IN A SPONTANEOUS MURINE MELANOMA MODEL

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A tumor is not made up of cancer cells alone. It consists of an elaborate stroma made of fibroblasts, endothelial cells and immune cells. But most studies on tumor microenvironment have been carried out in transplanted tumor models which only allows a limited time for establishing interactions with the immune system. We therefore constructed a transgenic mouse (MT-ret-AAD) which develops spontaneous skin tumors of melanocytic origin. In this model, primary eye and skin tumors metastasize to internal organs such as the reproductive tract, lungs, gut and brain. As the tumors develop over a relatively long time (~9weeks), there is enough time for the tumor and the immune systems to establish rather elaborate interactions. Inherent in realistic spontaneous tumor models, T cell analysis is usually made difficult due to the tumor antigen being unknown. On the other hand many transplanted tumor models use surrogate tumor antigens (e.g. ovalbumin or viral antigens) which inaccurately reflect the usually poor immunogenicity of natural, self derived tumor antigens. We circumvent this problem by expressing a chimeric HLA-A2 molecule in our mice so that T cell responses specific for tumor antigens that have been identified in human patients can be analyzed. We found that all mice developing tumors mount a strong immune response to melanoma differentiation antigens. But this response does not affect the development of skin tumors.(Lengagne et al, 2008) Skin tumors do not escape cytotoxic T cell recognition by immune editing 1) We do not detect any melanoma-differentiation antigens being down-regulated in the tumors. 2) CD8 depletion does not affect skin tumor onset, growth or number. 3) Transplants of skin tumors into syngeneic mice are rejected but not when they are transplanted into nude mice. Further studies are being carried out to detect and analyse the occurrence of internal metastases. This is hindered by the small size of these tumors and their unpredictable appearance in the internal organs. We have constructed a new transgenic mouse, Melucie, in which tumors express the firefly luciferase and can be detected via bioluminescence. In the MT-ret-AAD mice, immune cells constitute 1.5% – 4% of the entire tumor. A large percentage of these cells are myeloid in nature (i.e. dendritic-like, monocyte-like, macrophage-like and granulocyte-like). Lymphoid cells like CD4 and CD8 T cells, B cells and NK cells have also been detected, albeit in lesser numbers. Although these cell types appear to be present in all tumors, certain subsets seem to vary in number from tumor to tumor even in the same mouse. We are currently analyzing the transcriptome of these immune cells and trying to identify which factors affect the nature and polarization of these infiltrates. By comparing the transcriptome data with the phenotypic characteristics of the tumor, we hope to further understand the role of these immune cells in the development of the tumor.

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Macrophages Selective Immune Activation with BCG-Indometacin kills cancer cells

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Peritoneal macrophages in vitro adherent cells from Swiss mice bearing subcutaneous Ehrlich AK are unable to ingest the AK cells while from normal animals 105 in active and up to 95% from immunized with BCG intradermic (8-10 injections of 0,1 ml. in saline-0,8-3,2 10 to the fifth CFU Istituto Vaccinogeno di Milano) and cured (uptake up to 3 cancer cells for each macrophage) Imperato 1969,70,71).From 1973 to 1994 124 patients(64 males and 60 females age from 46 to 75) with intestinal cancer C with peritoneal diffusion after surgery and first line chemotherapy have been treated with BCG and Indometacine 50 mg pro die. BCG in saline(BCG Copenaghen 0,1 ml –0,8-3,2-10 to do sixth -to do seventh)has been given by INTRAEPITELIAL (at deltoids end to do third upper of the legs) every other day for 4-6 weeks and after every week. The Immune Selective Activation has been measured by the diameter of erythema infiltrate (EI),WBC, lymphocytes and monocites count, PHA activation in vitro. 16-36 (average 24) months good survival correlate with 20 mm EI(68%), 8-14 (average 12) 8 mm (20%). Synergic Reciprocal therapeutic effects produced by the interaction of PG2 inhibition due to the Indometacine and the Selective Immune Activation of macrophages .All patients had a good improvement of the live quality. The E:I of 12 mm minimum correlate with increase of WBC, lymphocytes (30-50%) and monocytes (30-70%) and PHA blast transformation from 5 to 20% (microscopic reading). The Intraepitelial Immune Selective Activation with BCG-Indometacine kill and eliminate cancer cells and used before and after (at proper intervals) enhance and multiply the Efficacy-Efficiency of any other therapy (surgery, chemo, radio, hormone , antibodies …….) SINTERAPIA. I applied the Synergism Selective Immune – Radio 50% - Immuno to patients with advanced inoperable lung cancer and published the results at National Congress of Oncology-Bari-1980 and at First National Congress of Medical Oncologist (AIOM)-Rome-1999. I obtained out of 52 patients the 20% cured at 5 years. The results correlate with the parameters described above. The Proper Alliance of the described Immune Therapy with All the others Immunotherapies and any other therapy MULTIPLY the Efficacy-Efficiency with reciprocal better therapeutic and preventive better results already.

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THE Gr-1 (RB6-8C5) ANTIBODY IS NOT DEPLETING BUT INDUCES DIFFERENTIATION OF MACROPHAGES AND THEREBY ABLATES MYELOID-DERIVED SUPPRESSOR CELL ACTIVITY

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The Gr-1 (RB6-8C5) aby has been described to deplete neutrophilic granulocytes but also myeloid-derived suppressor cells (MDSC) after injection into mice. The aby binds with high affinity to the Ly6G molecule and a lower extent to Ly-6C. Here we show that the Gr-1 aby is not depleting but instead remained attached to the cell surface for at least 4 days in the bone marrow and spleen. This sustained binding induced upregulation of MHC I, CD115 (MCSF-R), F4/80 and asialo-GM1 but not CD11c on Ly-6G+ cells, indicating the differentiation of macrophages but not dendritic cells. As a consequence early myeloid Gr-1+ CD11b+ MDSC lost their suppressive capacity during this 4-day-period. While in bone marrow most Gr-1+ cells appear not depleted by Gr-1 aby in vitro or after injection, the effect on differentiated neutrophils in the spleen remains unclear to date due to their high turnover. Thus, the reported depleting effects of the Gr-1 aby in infection or tumor models may therefore be rather due to the functional deviation of Gr-1-expressing cells into macrophage differentiation and thereby blocking dendritic cell and granulocyte generation. Together our data suggest a functional role of the Ly-6G molecule in the myeloid lineage differentiation by Gr-1 aby occupation but not cell depletion.

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Molecular characterization of Tie2-expressing monocytes (TEMs): relationship with tumor-associated macrophages

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Increasing evidence is accumulating that resistance to antiangiogenic therapy based on VEGF neutralization may occur, and that myeloid cells that infiltrate tumors may contribute to this effect, perhaps by producing alternate proangiogenic growth factors. Our studies are aimed at understanding the interactions between innate immune cells and the vascular system, in order to identify alternative targets for antiangiogenic therapy. We studied the contribution and functional importance of myeloid-lineage cells in the process of tumor angiogenesis. We showed that a subset of monocytes that express the angiopoietin receptor Tie2 play an important role in tumor angiogenesis. Selective depletion of these Tie2-expressing monocytes (TEMs) in tumor-bearing mice inhibited tumor angiogenesis and growth, suggesting that they might regulate angiogenic processes in tumors by providing paracrine support to nascent blood vessels. TEMs have also been recently identified in human blood and tumors. Yet, a detailed molecular analysis of TEMs is still lacking, and their lineage-relationship with other tumor-infiltrating myeloid populations remains to be clarified. We have developed protocols to isolate highly purified TEMs and tumor-associated macrophages (TAMs) from mouse mammary carcinomas. We compared the expression profile of TEMs with that of TAMs using qPCR-based arrays interrogating ~300 genes in three different biological samples. We found that both TEMs and TAMs express markers previously associated with M2-polarized macrophages. These include molecules involved in tissue-remodeling/angiogenesis, such as canonical proangiogenic factors, proteinases and scavenger receptors. Despite the global similarity between TEMs and TAMs, statistical modeling of the gene expression data revealed several differentially expressed genes. Notably, classical proinflammatory (M1) molecules were significantly downregulated in TEMs, whereas prototypical M2 markers were strongly upregulated. The RNA data were confirmed by protein expression analysis in two mouse mammary tumor models, including tumors spontaneously developing in MMTV-PyMT transgenic mice. Intriguingly, the M2-skewed phenotype of TEMs was already expressed by cells isolated from the circulation, suggesting that TEMs may have inherent protumoral and proangiogenic activity among circulating monocytes. Our data support the provocative concept that TEMs and TAMs observed in tumors may derive from developmentally distinct monocyte subsets.

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The novel non-toxic tellurium compound SAS blocks angiogenesis by inhibition of cell migration and adhesion: interference with αvβ3 activity.

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A novel Te(IV) small molecule, octa-*O*-bis-(*R,R*)-Tartarate Ditellurane (SAS), is shown in the present study to have a direct anti-angiogenic activity *in vivo* and *in vitro*. This was expressed in increased inhibition of endothelial cell migration induced by angiogenic stimuli in various models, including Boyden chamber, "scratch" wound closure, single cell analysis by time lapse photography and the CAM (Chorio-Allantoic Membrane) assay. The Te(IV) chemistry of SAS allowed it to interact with thiols enabling it to inhibit the activity of specific proteins like papain, in which a particular redox status of cysteine is essential to their biological activity. This redox attribute of SAS is used here to functionally inhibit the activity of β integrins, being recently suggested to be controlled directly by a redox site in the extracellular domain. Indeed, SAS-mediated integrin inhibitory activity is demonstrated in this study since it inhibited cell attachment via β integrins in which the cysteine redox site resides but not α integrins subunits. This chemical activity was associated with SAS ability to diminish endothelial cell adhesion to the ECM proteins, fibronectin and the bioactive laminin-derived peptide, via blocking of αVβ3 integrin binding. Furthermore, signaling events that follow integrin binding were substantially inhibited. More importantly, interference with αVβ3 binding by SAS was associated with both its direct anti angiogenic property on endothelial cells and its direct effect on tumor cells. The integrated results emphasize the potential use of the tellurium non-toxic molecule, SAS, as a new class of anti-angiogenic, anti-cancer compound.