

MEETING GREMI 2008

TH17-DERIVED CYTOKINES : NEW KIDS ON THE BLOCK OF INFLAMMATION

December, 12th, 2008 ; Institut Pasteur, Paris, France

Functions of NOD2 signaling in the induction of Th17 responses

John BAKER, Rachel Cooney, Oliver Brain, Dilair Baban, & Alison Simmons

MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Headington, Oxford. OX3 9DS. United Kingdom

Crohn's Disease (CD) is a debilitating condition of chronic Th17-dominated gastrointestinal inflammation. Many CD susceptibility genes function in innate immune processes including signaling and autophagy; some, like IL23R, function in the Th17 pathway. CD-associated mutations of NOD2, a Dendritic Cell (DC)-expressed intracellular pattern recognition receptor, result in impaired NF-kappaB induction following triggering. It is unclear how this defect translates into the inflammatory Th17 response of CD. DCs are prototypic antigen presenting cells responsible for priming adaptive immune responses; NOD2 activation in DCs results in preferential expansion of Th17 cells through mechanisms as yet unknown. CD-associated gene ATG16L1 encodes an autophagy pathway protein essential for autophagosome formation. Autophagy is involved in cellular bacterial handling, and MHC II-mediated antigen presentation.

Aims: To dissect the roles of NOD2 in transcriptional regulation and antigen presentation in human primary DCs.

Methods: DCs were derived from human PBMC by CD14⁺ selection and culture in IL-4 and GM-CSF. DC transcriptional responses to NOD2, TLR2, and dual stimulation were characterised by microarray analysis, and validated by ELISA. Autophagy induction was observed by immunoblotting to demonstrate autophagy-associated LC3 isoform shifts, and quantified by confocal and electron microscopy. Knockdown of NOD2 and autophagy pathway components in DCs was achieved by AMAXA siRNA transfection.

Results: NOD2 activation induces transcriptional changes in DCs, and modulates transcriptional responses to TLR2. Strongly induced genes include CCL20, a chemokine mediating chemoattraction of CCR6⁺ Th17 cells, HLA-DR, and other antigen presentation and immune regulatory genes. Further, NOD2 stimulation induces autophagosome formation in DCs, inhibited by knockdown of NOD2, ATG16L1, and autophagy pathway components. Successful autophagosome formation is required for MHC II upregulation. CD patient DCs expressing NOD2 variants are defective in NOD2-induced transcription and autophagy.

Conclusions: NOD2 activation induces transcriptional changes and modulates TLR2 responses, enhancing MHC II and co-stimulatory molecule upregulation. NOD2-induced MHC II upregulation is dependent on NOD2-mediated autophagy induction. Further, NOD2 activation enhances production of TH17-chemoattractant CCL20. These responses, impaired in variant NOD2-expressing CD-patient DCs, may influence the nature and context of antigen presentation, and favour the induction of TH17 responses through preferential recruitment of CCR6-positive CD4 cells to priming DCs.

POSTER 1

MEETING GREMI 2008

TH17-DERIVED CYTOKINES : NEW KIDS ON THE BLOCK OF INFLAMMATION

December, 12th, 2008 ; Institut Pasteur, Paris, France

Effect of the neutralization of IL-17A on the immune response to M. tuberculosis infection

Danielle FRECHES¹ ;Hannelie Korf¹, Catherine Uyttenhove², Jacques Van Snick², Muriel Moser³, Kris Huygen¹ and Marta Romano¹

¹Scientific Institute for Public Health, Pasteur Institute, Brussels, Belgium; ²Christian De Duve Institute of Cellular Pathology, Université Catholique de Louvain, Brussels, Belgium;³Institut de Biologie et Médecine Moléculaires, Université Libre de Bruxelles, Gosselies, Belgium

Tuberculosis is caused by M. tuberculosis and remains a major health problem. Development of a more effective vaccine is essential but requires a better understanding of the immune response against M. tuberculosis. Little is known about the role of IL-17A in M. tuberculosis control and the published data have been controversial. In this study, we have analyzed the effect of the neutralization of IL-17A on the immune response induced by M. tuberculosis. For that purpose, DBA/2 mice were vaccinated with an anti-IL-17A-OVA auto-vaccine [1] and subsequently infected with M. tuberculosis via the intratracheal route. Preliminary results have shown no differences in bacterial replication between the control and the IL-17A-neutralized group. Analysis of total BAL cells by cytopspin showed that 2 and 4 weeks, but not 13 weeks after infection, less neutrophils were infiltrating the lungs of IL-17A-neutralized mice compared to control groups. RT-PCR analysis of total BAL cells and lung CD4+ T cells showed increased expression levels of IL-17A and IFN-gamma in the IL-17-auto-vaccine treated group 13 weeks after infection. Histological analysis of the lung tissue is in progress.

Acknowledgements: This work is supported by FWO Vlaanderen - Krediet aan navorsers to MR. n°1.5.230.08. DF holds a FRIA bursary

Uyttenhove, C. and J. Van Snick, Development of an anti-IL-17A auto-vaccine that prevents experimental auto-immune encephalomyelitis. Eur. J. Immunol., 2006, 36(11): p. 2868-2874.

MEETING GREMI 2008

TH17-DERIVED CYTOKINES : NEW KIDS ON THE BLOCK OF INFLAMMATION

December, 12th, 2008 ; Institut Pasteur, Paris, France

The role of IL-17A in immune responses

Eve HORNSBY

Lab 312, National Institute for Medical Research, The Ridgeway, London NW7 1AA

IL-17A is a pro-inflammatory cytokine secreted by haematopoietic cells that amplifies the innate response to pathogens. As well as its beneficial role in the host response to bacterial and fungal pathogens, IL-17A has been associated with the development of autoimmune responses in both mice and humans and therefore may be one of the molecules providing a link between infection and autoimmunity. IL-17A was first cloned in 1993, however it has received significant attention recently due the description of the Th17 lineage of CD4+ effector cells. These cells have been described to be important in the immune response against various bacterial and fungal infections as well as being involved in the development of autoimmunity, as observed in the mouse models Experimental Autoimmune Encephalomyelitis (EAE) and Collagen Induced Arthritis (CIA). However, IL-17A is secreted by many other cells of the immune system. In particular NK cells, $\gamma\delta$ T cells, CD8+ T cells and B cells have also been reported to express the molecule. In order to determine some of the mechanisms by which IL-17A is involved in immune responses and to investigate the possibility that IL-17A may be responsible for connecting the immune response to an infectious agent and the development of autoimmunity, I am investigating the different cell types expressing IL-17A after pathogen stimulation and also in the course of autoimmunity in mice.

MEETING GREMI 2008

Th17-DERIVED CYTOKINES : NEW KIDS ON THE BLOCK OF INFLAMMATION

December, 12th, 2008 ; Institut Pasteur, Paris, France

The role of IL-17A during infection with *Leishmania major*.

C. RONET, C. Finsterwald, Y. Hauyon-La Torre, P. Launois

The WHO Immunology Research and Training Center, Department of Biochemistry, University of Lausanne, Epalinges, Switzerland.

Some features of the cutaneous pathologies observed in patients with cutaneous leishmaniasis can be reproduced in the murine model of infection with *Leishmania major* (*L. major*). After subcutaneous injection of parasites in footpad, C57BL/6 mice are resistant to infection whereas BALB/c mice are susceptible. Resistance and susceptibility to the infection were related to the development of polarized T helper (Th) 1 and Th2 response. Recently Th17 lineage of CD4⁺ T helper lymphocytes, secreting the cytokine IL-17, has been characterized as an independent lineage from Th1 and Th2. The aim of this study is to determine if Th17 participates in the immune response against *L. major*.

IL-17^{-/-} BALB/c and C57BL/6 mice were infected with *L. major* promastigotes. (strain LV39, 3x10⁶ in the footpad).

No difference in the lesion size was observed in IL-17^{-/-} and wild type (WT) BALB/c mice. Nevertheless parasite load and IL-4 production by *L. major* restimulated draining LN cells were lower in IL-17^{-/-} BALB/c than in WT mice.

IL-17^{-/-} C57BL/6 are more resistant to infection than WT C57BL/6 mice, have a lower parasite load and develop a reduced Th1 cell response.

Phenotype of IL-17 producing cells have been determined. Preliminary results showed that the main producing cells are TCR α CD4⁺ and CD4⁻CD8⁻ T cells.

Altogether these results suggest a discrete role of Th17 cells on the course of infection with *L. major*.

MEETING GREMI 2008

TH17-DERIVED CYTOKINES : NEW KIDS ON THE BLOCK OF INFLAMMATION

December, 12th, 2008 ; Institut Pasteur, Paris, France

Protective immunity to systemic infection with *Salmonella enterica* in the absence of IL-12 is associated with IL-23-dependent IL-22 but not IL-17

Silke M. Schulz¹, Gabriele Koehler², Nicole Schuetze¹, Jens Knauer¹, Reinhard K. Straubinger¹, Alissa A. Chackerian³, Ellen WITTE, Kerstin Wolk⁴, Robert Sabat, Yoichiro Iwakura⁵, Christoph Holscher⁶, Uwe Mueller¹, Robert A. Kastelein³, and Gottfried Alber¹

¹Institute of Immunology, College of Veterinary Medicine, University of Leipzig, Leipzig, Germany

²Gerhard Domagk Institute of Pathology, University of Münster, Münster, Germany

³Discovery Research, Schering-Plough Biopharma, Palo Alto, USA

⁴Interdisciplinary Group of Molecular Immunopathology, Dermatology/Medical Immunology, University Hospital Charité, Berlin, Germany

⁵University of Tokyo, Tokyo, Japan

⁶Research Center Borstel, Borstel, Germany

IL-12 is essential for protective T cell-mediated immunity against *Salmonella* infection. To characterize the role of the IL-12-related cytokine IL-23, wild-type C57BL/6 (WT) and p19^{-/-} mice were infected systemically with an attenuated strain of *Salmonella enterica* serovar Enteritidis (S. Enteritidis). In the absence of IL-23, infected animals showed strongly reduced IL-17A and IL-22 but similar IFN- γ production, and had a compromised delayed-type hypersensitivity reactivity compared to WT mice. Nevertheless, IL-23-deficient mice controlled S. Enteritidis infection similarly to WT mice. Hence, although IL-23 was required for T-cell effector responses, it was not essential for the protection against systemic S. Enteritidis infection when IL-12 was present. To analyze the role of IL-23 in the absence of IL-12, low doses of S. Enteritidis were administered to p35^{-/-} mice (lacking IL-12), p35/19^{-/-} mice (lacking IL-12 and IL-23), p35/40^{-/-} mice (lacking IL-12, IL-23, and homodimeric p40), and p35/IL-17A^{-/-} mice (lacking IL-12 and IL-17A). We found survival of p35^{-/-} and p35/IL-17A^{-/-} mice, whereas p35/19^{-/-} and p35/p40^{-/-} mice died within 3 - 6 weeks and developed liver necrosis. This indicates that IL-23 but not homodimeric IL-12p40 is required for protection which, surprisingly, is independent of IL-17A. Moreover, protection was associated with IL-22 but not IL-17F or IL-21 expression or with neutrophil recruitment. Finally, anti-IL-22 treatment of S. Enteritidis-infected p35^{-/-} mice resulted in liver damage. In conclusion, simultaneous abrogation of IL-12 and IL-23 causes mice to become hypersensitive to systemic infections with S. Enteritidis and IL-23-dependent IL-22 but not IL-17 production is associated with protection against systemic infection with S. Enteritidis in the absence of IL-12.

MEETING GREMI 2008

TH17-DERIVED CYTOKINES : NEW KIDS ON THE BLOCK OF INFLAMMATION

December, 12th, 2008 ; Institut Pasteur, Paris, France

Protective immunity to *Streptococcus pneumoniae* is related to IL-17 expression

Jean-Claude SIRARD¹, Laurye Van Maele¹, Juan M. Marqués², Arndt Benecke³, José A. Chabalgoity²

¹ INSERM U801, Institut Pasteur de Lille, Equipe d'Immunité Anti-Microbienne des Muqueuses

² Laboratory for Vaccine Research, Department of Biotechnology, School of Medicine- Universidad de la República, URUGUAY

³ Institut des Hautes Etudes Scientifiques, CNRS USR3078, Lille

Streptococcus pneumoniae or pneumococci are major bacterial agents of respiratory tract infections, with 10 million deaths per year worldwide. Our aim is to determine immune effectors and mechanisms involved in mucosal innate and adaptive protection against pneumococcus. We used an experimental model based on intranasal administration of the invasive serotype 1 *S. pneumoniae* that causes acute pneumonia and death in mice within 72h. We found that intranasal administration of sublethal doses of bacteria on day 1 elicits 100% protection against subsequent challenge with a lethal dose of the same strain one week later. Based on these results, we have conducted transcriptional profiling studies in whole lung to assess the protective signature. Microarray analysis of lungs obtained 24 hours after challenge showed differential gene expression between the protected and the control animals. Most of the altered biological processes are related to immunity and defense. The protective signature was associated to the increase of lung IL-17A and IL-17F expression. The protective mechanisms as well as IL-17 expression were dependent on the Toll-like receptor adaptor molecule MyD88. Similar correlations were associated in the natural resistance of naïve mice to the *S. pneumoniae* infection. Our data suggest a role for IL-17 in innate and adaptive defenses to acute pneumonia triggered by pneumococcus. These observations could be relevant to define novel strategies of intervention.

MEETING GREMI 2008

TH17-DERIVED CYTOKINES : NEW KIDS ON THE BLOCK OF INFLAMMATION

December, 12th, 2008 ; Institut Pasteur, Paris, France

Stability and plasticity of Th17 cells in vitro and in vivo

**Annegret TAUBNER¹, Maria Lexberg², Anna Förster³, Inka Albrecht², Anne Richter³,
Sylvia Heink¹, Oliver Frey¹, Andreas Radbruch², Hyun-Dong Chang², Thomas
Kamradt¹**

¹Institut für Immunologie, Universitätsklinikum, Jena, Germany

²Deutsches Rheumaforschungszentrum, Berlin, Germany

³Miltenyi Biotec GmbH, Bergisch Gladbach, Germany

Different subsets of Th cells have been described based their expression of typical cytokines. Activated T cells are able to re-express their cytokine pattern which is known as memory.

The stability and also the plasticity of Th17 cells in vivo and in vitro is still unclear. To investigate the stability and plasticity of Th17 cells we sorted naïve T cells (CD4+CD62L+) cultured in vitro for one week in the presence of IL-6, TGF-beta, anti-IL-4, anti-IFN-gamma and re-cultured the cells under Th1 conditions. Continued IL-17 expression of in vitro differentiated Th17 cells depended on TGF-beta and IL-6. In contrast, addition of IL-12 converted Th17 cells into IFN-gamma producing T cells, respectively.

For analysis of Th17 cells ex vivo we used the newly developed IL-17 cytokine secretion assay for the isolation of viable Th cells secreting IL-17.

We sorted IL-17+ T cells from mice, which had been immunized with MOG-peptide to induce experimental autoimmune encephalomyelitis (EAE). The IL-17+ T cells from mice at day 7 post immunization, maintained their IL-17 expression upon subsequent culture in vitro under Th17 polarizing conditions similar to the sorted IL-17+ T cells from unimmunized aged mice (Lexberg et al., Eur J Immunol, 2008). Interestingly, 45 % of the IL-17 producing T cells from immunized mice co-expressed IFN-gamma under Th17-conditions which was higher than expected for random co-expression. The fraction of IL-17 producers from immunized mice was reduced by approximately 50%, after one week of in vitro culture under Th1-, Th2- or non-polarizing (Th0-) conditions. A significant fraction of the cells converted into IFN-gamma producing T cells in vitro.

Our results show that newly activated IL-17+ T cells in secondary lymphoid organs of MOG immunized mice are not stably imprinted for the re-expression of IL-17.

MEETING GREMI 2008

TH17-DERIVED CYTOKINES : NEW KIDS ON THE BLOCK OF INFLAMMATION

December, 12th, 2008 ; Institut Pasteur, Paris, France

A critical role for TGF- β , IL-23 and pro-inflammatory cytokines in driving and modulating human TH-17 responses

Elisabetta VOLPE^{1,2}, Nicolas Servant^{3,4,5}, Raphaël Zollinger^{1,2}, Sofia I. Bogiatzi^{1,2}, Philippe Hupé^{3,4,5,6}, Emmanuel Barillot^{3,4,5}, Vassili Soumelis^{1,2}

¹ Institut National de la Santé et de la Recherche Médicale U653

² Institut Curie, Laboratoire d'Immunologie Clinique, Paris, France

³ Bioinformatique, Institut Curie, Paris, F-75248 France

⁴ Institut National de la Santé et de la Recherche Médicale, U900, Paris, F-75248 France

⁵ Ecole des Mines de Paris, ParisTech, Fontainebleau, F-77300 France

⁶ CNRS, UMR144, Paris, F-75248 France

T helper 17 cells (TH-17 cells) were recently described as a T helper (TH) cell subset distinct from T helper type 1 (TH1) and TH2 cells, with specific roles in anti-microbial defence and auto-immunity. The environmental factors driving human TH-17 cell responses as well as the balance between protective or pathogenic role of Th17 cell responses is of particular interest. We investigated about the factors driving and modulating human TH-17 differentiation. Using a systematic approach combining experimental and computational methods, we show here that TGF- β , IL-23 and pro-inflammatory cytokines (IL-1 β and IL-6) were all essential for human TH-17 differentiation. In this study we show that TH-17-promoting cytokines differentially modulate individual TH-17-derived cytokines, such as IL-17, IL-21, IL-22, or IL-6, as well as the global TH-17 cytokine profile. In particular, TGF- β was critical and its absence induced a shift from TH-17 to a TH1-like profile. Our results shed new light on the regulation of human TH-17 differentiation, and provide a framework for the global analysis of TH responses.

MEETING GREMI 2008

TH17-DERIVED CYTOKINES : NEW KIDS ON THE BLOCK OF INFLAMMATION

December, 12th, 2008 ; Institut Pasteur, Paris, France

Patients with acne inversa show a relative deficiency in the cutaneous expression of antimicrobial peptides which may be due to a relative IL-22 deficiency and may be responsible for this chronic skin disorder

Kerstin Wolk, Katarzyna NASILOWSKA¹, Conny Hoefflich², Ellen Witte¹, Stefanie Kunz¹, Sylke Schneider³, Hans Joachim Roewert³, Hans-Dieter Volk², Wolfram Sterry³, Robert Sabat¹

¹Interdisciplinary Group of Molecular Immunopathology, Dermatology/Medical Immunology, University Hospital Charité, Berlin, Germany

²Institute of Medical Immunology, University Hospital Charité, Berlin, Germany

³Department of Dermatology, University Hospital Charité, Berlin, Germany

Acne inversa is a chronic skin disease with unknown pathogenesis. In contrast to some other cutaneous inflammatory disorders such as psoriasis, AI patients frequently suffer from bacterial infections of the skin lesions. We demonstrate for the first time that the expression of antimicrobial peptides (AMPs), a major first line defense against bacterial skin invasion, were significantly diminished in AI lesions compared to psoriatic lesions. Furthermore, the AI lesions contained significantly lower expression levels of IL-22, IL-20, and IL-19, whereas the expression of IL-17A, IFN- γ , and IL-1 β did not differ between psoriatic and AI lesions. In reconstructed human epidermis IL-22 and IL-20 upregulated the expression of diverse AMPs such as b-defensin 2, b-defensin 3, and the S100 proteins S100A7, S100A8, and S100A9. Additionally, IL-22 induced cutaneous b-defensin expression after application in BALB/c mice. In contrast to IL-22, IFN- γ did not upregulate the expression of b-defensins in reconstructed human epidermis. Histological and immunohistological analyses showed comparable immune cell infiltrations in psoriatic and IA lesions. Interestingly, IL-10 was higher expressed in AI lesions compared to psoriatic skin, and IL-10 inhibited the production of IL-22 in T-cells. In conclusion, the relative AMP deficiency found in AI skin lesions may be responsible for the bacterial infections frequently seen in AI, and may be caused by the relative cutaneous IL-22 deficiency. A reason for that IL-22 deficiency may lie in the presence of high amounts of IL-10 able to inhibit the T-cell IL-22 production.