XXXVII Annual Meeting of the Portuguese Society of Immunology

“Portrait of the Immune System: History and Perspective”

Instituto Gulbenkian de Ciência

November 29–30, 2011

Abstract book

Organizers:
Jocelyne Demengeot
Ivo Marguti
Carlos E. Tadokoro
Jorge Carneiro
Thiago Carvalho
Welcome to the XXXVII SPI Annual Meeting!
This year's Reunion of the Portuguese immunological community will start with an exceptional session shared with the symposium "Jerne100: a century of Niels K Jerne", a gathering of the colleagues, associates, and friends of one of the most remarkable and intemporal figures of Immunology. In addition to the opportunity to present and discuss our work among ourselves we will have the unique opportunity to mingle with outstanding researchers who shaped immunology as we know it.

The organizers

Program Outline

Tuesday, November 29, 2011

09h00–09h30: Registration
09h30–13h30: Joint session with "Jerne100: Waiting for the end."
13h30–15h00: Lunch & Posters
15h00–15h45: Keynote 1: Harald von Boehmer
15h45–16h30: Oral communications
16h30–17h00: Coffee break & Posters
17h00–17h45: Keynote 2: Jonathan Sprent
19h00: SPI General Assembly
20h30: Conference Dinner

Wednesday, November 30, 2011

09h00–09h45: Keynote 3: Bjarne Bogen
09h45–10h30: Oral communications
10h30–11h00: Coffee break
11h00–11h45: Keynote 4: Lucien Aarden
11h45–12h30: Keynote 5: Jack Kettman
12h30–14h00: Lunch & Posters
14h00–14h45: Keynote 6: Louis du Pasquier
14h45–15h30: Keynote 7: Jean–Claude Weill
15h30–16h00: Coffee break
16h00–17h00: Oral communications
17h00–17h45: Keynote 8: Matthias Wabl
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1.1. **Harald von Boehmer**: "Proliferation versus Differentiation in alfa beta T cell development"

**Affiliation**: Dana–Farber Cancer Institute, Department of Cancer Immunology and AIDS

1.2. **Jonathan Sprent**: “Expanding T cell subsets in vivo with IL-2/MAb complexes”

**Affiliation**: Garvan Institute of Medical Research, Immunology, and Inflammation Research Program

1.3. **Bjarne Bogen**: “B cell diseases caused by chronic help from Idiotype-specific T cells”

**Affiliation**: Centre for Immune Regulation, University of Oslo

1.4. **Lucien Aarden**: “Not supplied”

**Affiliation**: Sanquin Blood Supply Foundation, Department of Autoimmune Diseases

1.5. **Jack Kettman**: "A History, so far"

**Affiliation**: Professor University of Texas Southwestern Medical School, Department of Immunology

1.6. **Louis du Pasquier**: "How the amphibian Xenopus became a tool for immunologists"

**Affiliation**: Universität Basel, Zoological Institute

1.7. **Jean–Claude Weill**: "Diversity is generated with diversity"

**Affiliation**: Université René Descartes, Faculté de Médecine Necker–Enfants Malades

1.8. **Matthias Wabl**: “Autoimmunity and Cancer”

**Affiliation**: University of California San Francisco
1.1. Harald von Boehmer: "Proliferation versus Differentiation in alfa beta T cell development"

**Affiliation:** Dana–Farber Cancer Institute, Department of Cancer Immunology and AIDS

**Abstract not supplied.**
Notes:
1.2. Jonathan Sprent: “Expanding T cell subsets in vivo with IL-2/MAb complexes”

Affiliation: Garvan Institute of Medical Research, Immunology, and Inflammation Research Program

Authors: J Sprent, O Boyman, K Webster, C Kreig, S Letourneau, C D Surh.

Abstract

Past work showed that the biological activity of cytokines, especially IL-2, in vivo can be augmented by association with specific mabs. Injection of IL-2 bound to certain IL-2 mabs, eg S4B6, leads to selective expansion of cells expressing low-affinity IL-2R, notably memory CD8 cells and NK cells. IL-2/S4B6 complexes have more potent anti-tumor activity in vivo than soluble IL-2 and, unlike IL-2, are relatively non-toxic and fail to cause expansion of suppressive CD4 T regulatory cells (Tregs). By contrast, IL-2 complexed with other IL-2 mabs such as JES6-1 induces selective expansion of cells expressing high-affinity IL-2R, notably Tregs and NKT cells. The use of IL-2/ES6-1 complexes to induce prolonged tolerance of tissue allografts in mice without the need for immunosuppression will be discussed.
1.3. Bjarne Bogen: “B cell diseases caused by chronic help from Idiotype–specific T cells”

Affiliation: Centre for Immune Regulation, University of Oslo

Abstract:
It was established more than 20 years ago that B cells constitutively process their B cell receptor (BCR) and present idiotypic (Id) peptides derived from the variable (V) regions on their MHC class II molecules to T cells. It was further demonstrated that while T cells are tolerant to abundant germline-encoded V region sequences, they can respond to rare Id–peptides that depend on somatic mutations or N–region diversity unique to each B cell. By establishment of a paired transgenic system, collaboration between Id+ B cells and Id–specific CD4+ T cells could be studied. Such Id–driven T–B collaboration is unlinked since the BCR may recognize e.g. an autoantigen while the TCR recognizes Id/MHC II complexes on the B cell’s surface. Moreover, the collaboration can be chronic since the autoantigen and the Id–peptide are continuously present. This is a dangerous constellation that could result in chronic B cell proliferation and differentiation, and disease. Indeed, during the last few years it has been demonstrated that chronic Id–driven T–B collaboration can result in autoimmune disease with SLE–like features and development of B lymphomas. These results obtained in a transgenic mouse model have recently been extended to patients with chronic lymphocytic lekemia. Apart from the above, if time allows, a recently described doubly Ig knock–in mouse with an anti–Id BCR will be described.
Notes:
1.4. Lucien Aarden: “Not supplied”

Affiliation: Sanquin Blood Supply Foundation, Department of Autoimmune Diseases

Abstract not supplied.
Notes:
1.5. Jack Kettman: "A History, so far"

Affiliation: Professor University of Texas Southwestern Medical School, Department of Immunology

Abstract:
A brief history of my travel through the normal Academic Path with side trips that proved very interesting. Enterprise was encountered, exploited and remains a continuing saga. Luminex and bead-based assays explained.
Notes:
1.6. Louis du Pasquier: "How the amphibian Xenopus became a tool for immunologists"

Affiliation: Universität Basel, Zoological Institute
Abstract not supplied.
Notes:
1.7. Jean-Claude Weill: "Diversity is generated with diversity"

**Affiliation:** Université René Descartes, Faculté de Médecine Necker–Enfants Malades

**Abstract:**
We will show that the pre-immune B cell repertoire can be generated with different molecular and cellular strategies depending on the species. While mice and men have selected for an ongoing rearrangement process event taking place in bone marrow throughout life, chicken, sheep and rabbit use their gut associated lymphoid tissues in order to generate their pre-immune repertoire. B cell diversity is generated in these species during the first months of life by post-rearrangement molecular mechanisms such as gene conversion and/or hypermutation. Finally we will summarize what is known today about the molecular transactions underlying these two processes.
Endogenous retroelements – retroviruses and retrotransposons – make up almost half the genome in humans and mice. They replicate as part of the cellular DNA, but they also replicate with a semi-autonomous life cycle, which generates new RNA, protein, cDNA and mutations in both retroelements and cellular DNA. Although they are part of "self," their expression can thus lead to both autoimmunity and cancer. We will show examples of how retroelements cause severe hereditary autoimmune disease; and spontaneous autoimmune disease with associated cancer.
2. Oral presentations:
CD70 on dendritic and epithelial cells in the thymic medulla promotes CD4 Foxp3 regulatory T cell development via CD27. Julie C. Ribot¹,*, Jonathan M. Coquet²,*, Sabine Middendorp², Gerda van der Horst², Yanling Xiao², Nikolina Babala², Joana F. Neves³, Daniel J. Pennington³, Heinz Jacobs², Jannie Borst²,** and Bruno Silva–Santos¹**

Notes:
Clonal Analysis of V(D)J Rearrangement in Immunoglobulin Genes: Implications for Allelic Exclusion. Clara F.A. Pereira\textsuperscript{1}, Paulo Vieira\textsuperscript{2}, Vasco M. Barreto\textsuperscript{1}

Notes:
Generation of anti-inflammatory adenosine by leukocytes is regulated by TGF-β. Frederico S Regateiro, Duncan Howie, Kathleen F Nolan, Eleftherios I Agorogiannis, David R Greaves, Stephen P Cobbold, Herman Waldmann

Notes:
HIV-2 has a reduced ability to infect the human thymus. Helena Nunes-Cabaço, Rita Tendeiro, Russell B. Foxall, Rui S. Soares, Ana I. Pinheiro, Paula Matoso, Ana E. Sousa

Notes:
Host IL-10 production is responsible for neonatal mice susceptibility against Group B Streptococcus infections. Pedro Madureira\textsuperscript{1,2}, Elva Bonifácio Andrade\textsuperscript{1,2}, Liliana Oliveira\textsuperscript{1,2}, Adília Ribeiro\textsuperscript{1,2}, Margarida Correia–Neves\textsuperscript{3}, Patrick Trieu–Cuot\textsuperscript{4}, Manuel Vilanova\textsuperscript{1,2}

Notes:
Infected but not Bystander Dendritic Cells polarize CD4 T cells towards a non-protective T–bet INF–γ IL10 phenotype. Mariana Resende¹, Diana Moreira¹, Joana Cunha¹,², Bruno Neves³,⁴, Maria Teresa Cruz³,⁴, Anabela Cordeiro da Silva¹,⁵ and Ricardo Silvestre¹

Notes:
Lympho-stromal interactions decline the frequency of IL-7-expressing thymic epithelial cells independently of the establishment of the medullary epithelium compartment. Ana Rosalina Ribeiro¹, Heidi Schmid¹, Pedro Rodrigues¹, Alexandra Moreira¹, Alexandre do Carmo¹, James P. Di Santo², Nuno Lages Alves¹

Notes:
3. Abstracts (Oral Presentations + Abstracts)
Selective effects of NF-κB1 deficiency in CD4 T cells on the induction of Th2 and TFh cell functions

Karine Serre1, Elodie Mohr2, Adam F. Cunningham3, Roger Bird3, Mahmood Khan3, Cécile Benezech3, Jorge H. Caamaño3 and Ian C. M. MacLennan3

1 Instituto Molecular de Medicina, Unidade de Imunologia Molecular, Av. Professor Egas Moniz, 1649-028 Lisboa, Portugal. 2 Instituto Gulbenkian de Ciência, PT-2781-901 Oeiras, Portugal. 3 MRC Centre for Immune Regulation, the IBR, School of Immunity and Infection, University of Birmingham, Birmingham B15 2TT, United Kingdom

Abstract

NF-κB1-dependent signaling directs the development of CD4 Th2 cells during allergic airways inflammation and protective responses to helminth infection. Here, we show that IL-4 and IL-13 production is NF-κB1-dependent in ovalbumin-specific CD4 (OTII) T cells responding to alum-precipitated ovalbumin (alumOVA) immunization. More surprisingly, NF-κB1-deficiency in OTII cells also selectively impairs CXCR5 induction by alumOVA without affecting the upregulation of BCL6 and IL-21 mRNA. This leads to a functional TFh cell function impairment. Thus, there are fewer germinal center B cells in lymph nodes responding to alumOVA in T cell-deficient mice reconstituted with NF-κB1−/−OTII cells as opposed to NF-κB1+/+OTII cells, while plasma cell numbers are comparable. Similar results were obtained in CD40L-deficient mice, which fail to mount TD follicular and extrafollicular antibody responses and lack T cell-dependent Ig isotype switching. The selective effects of NF-κB1-deficiency on Th2 and TFh induction is not through altered expression of the Th2-associated transcription factors – GATA-3, cMaf and Ikaros. However, we found that Helios, which is usually associated with regulatory T cells, is strongly upregulated in CD4 Th2/TFh cells. Although its role in Th2/TFh CD4 T cells remains unknown, Helios expression is impaired in NF-κB1−/−CD4 T cells.

In contrast to the in vivo role of NF-κB1 in Th2 differentiation, IL-4 and IL-13 remained unimpaired in NF-κB1-deficient CD4 T cells cultured with TCR ligation in the presence of IL-4. This reinforces the evidence that a different signaling pathway operates in vivo Th2/TFh differentiation compared to in vitro. This work highlights the functional significance of NF-κB1 in CD4 T cells during responses to alum-precipitated proteins.
Serum immunoglobulins play a role in B cell homeostasis

Andreia C. Lino¹, Elodie Mohr¹, Jocelyne Demengeot¹

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Abstract

Total B cells numbers are remarkably stable in steady state, despite continuous production from the bone marrow. Similarly, soluble immunoglobulins (Ig) titers in serum remain stable at steady state. Here, we tested whether the tight homeostatic control of both B cells and their end product –Igs- results from a feed back mechanism whereby serum Ig would control B cell numbers. We compared the number of B cell in mice that lack secreted IgM (mS/-/- mice), or switched Ig (AID/-/-mice) or both and therefore lack all Igs (AID/-/-mS/-/- double mutant mice). Numbers of total B cells were slightly increased in the spleen of mS/-/- and AID/-/- mice when compared to those of WT animals, but three fold increased in the AID/-/-mS/-/- mice. The increase in total B cell numbers in mice devoid of Ig was due to an increase in activated cells, including marginal zone B cells, germinal center B cells and B-1 cells. While bone marrow cellularity was not affected by the lack of Ig, immature B cells expressed higher levels of IgM. Using mixed bone marrow chimeric mice we tested whether AID/-/-mS/-/- B cell phenotype could be corrected by Igs secreted by WT, µS/-/- or AID/-/- B cells. This approach demonstrated that secreted Igs control splenic B cell numbers, in particular marginal zone B cells and B-1 cells, but not GC B cells. Moreover, the analysis of AID/-/-µS/-/- mice raised in Germ-free conditions, established that secreted Igs limit the number of B cells activated by endogenous compounds. Together, these data establish that in steady state conditions, Ig exert a feedback regulation that participate in B cell homeostasis.
Generation of anti-inflammatory adenosine by leukocytes is regulated by TGF-β

Frederico S Regateiro, Duncan Howie, Kathleen F Nolan, Eleftherios I Agorogiannis, David R Greaves, Stephen P Cobbold, Herman Waldmann

Sir William Dunn School of Pathology, University of Oxford, United Kingdom

Abstract

Levels of anti-inflammatory extracellular adenosine are controlled by the sequential action of the ectonucleotidases CD39 and CD73, whose expression in CD4 T cells has been associated with natural regulatory T cells.

Our data show that CD73 expression on activated murine CD4 T cells is induced by TGF-β independently of Foxp3 expression. TGF-β induction of CD73 occurs at the transcriptional level and translates into gain of functional capacity to generate adenosine.

In the presence of AMP, CD73 induced by TGF-β in CD4 T cells generates adenosine able to suppress proliferation of activated CD4 T cells in vitro. These effects are contextual and opposed by pro-inflammatory cytokines.

CD73 is also upregulated by TGF-β in CD8 T cells, dendritic cells and macrophages, so providing an amplification mechanism for adenosine generation in tissue microenvironments. Together, these findings expose a novel anti-inflammatory role for TGF-β.
Role Of Haptoglobin And Its Polymorphism In Bronchial Asthma

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1Allergy Department-Hospital Santa Maria- CHLN – Lisbon- Portugal
2Genetic Department- Lisbon Medical School-Lisbon- Portugal

Abstract

Background: Haptoglobin (Hp), an alpha 2-sialoglycoprotein known to bind free hemoglobin (Hb), has been implicated in the modulation of Th1/Th2 response. The Hp locus is located at 16q22 chromosome, being in humans polymorphic for the ? chain, that leads to 3 genotype variants, Hp1-1, Hp2-1, Hp2-2.

Methods: 116 asthmatic patients were compared with a control group (n=50) in order to: 1) Evaluate different Hp genotype and allelic frequencies between the 2 groups; 2) Correlate the Hp genotype with serum Hp levels (intermediate phenotype/endotype); 3) Correlate Hp genotype and phenotype with asthma susceptibility/severity. Hp levels assayed by nephelometry and genotypes by PAGE. Statistical analysis was performed with PASW 18, establishing a significance level of p<0.05.

Results: Hp Allelic and genotype frequencies were not significantly different between groups. In asthma, differences were observed in Hp levels at the age-groups: <30 years presented lower Hp levels compared with ≥30 years (p<0.05). Additionally, Hp 2-2 asthmatics have lower levels of Hp when compared to Hp 2-1 and 1-1 (p<0.05). Different genotype distribution of Hp levels was only observed in the group ≥15 years (p<0.05). Hp 1-1 asthmatic patients presented an increased risk of 4.7 to be uncontrolled when compared to Hp2-2 patients (OR: 4.7; IC95% [1.012-21.891]). No differences in Hp levels between asthma and control group (137.83±51.4 mg/dL vs 123.92±51.36 mg/dL), however Hp 1-1 and 2-2 individuals presented statistical differences between groups, being the asthmatic patients those with lower levels of circulating Hp (p<0.05). In the control group, no differences were observed in Hp levels by genotype or age group (p>0.05).

Conclusions: Despite not having observed a prevalence of Hp allele 1 in asthmatics, that has been extensively associated with a Th2 profile, these patients had the highest levels of Hp and the higher risk of severe disease pointing to differences among groups that could be related.
Regulatory T cells are decreased in RHDV infection

Luzia Teixeira¹,², Raquel M. Marques¹,², Sara Cunha¹,², Artur P. Águas¹,², Paula G. Ferreira¹,²

¹ Departamento de Anatomia, ICBAS – Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Porto, Portugal. ²UMIB – Unidade Multidisciplinar de Investigação Biomédica, Porto, Portugal.

Abstract

Rabbit Haemorrhagic Disease Virus (RHDV) is responsible for an acute lethal disease (RHD, rabbit haemorrhagic disease) that kills European Rabbits (Oryctolagus cuniculus) worldwide. RHD is characterized by necrotizing hepatitis that induces a disseminated intravascular coagulation (DIC) and haemorrhage in several organs. In radical contrast with adult rabbits, young rabbits do not show any clinical signs of disease after being infected by RHDV. Therefore, looking for differences in the response of young and adult rabbits to RHDV infection may give us important clues to explain the lethality of this viral infection in adult rabbits and also contribute to understand the mechanism of fulminant liver failure.

In this work we focused in studying a subtype of T cells, naturally occurring CD4 Foxp3 regulatory T cells (Treg). In rabbits it was recently shown that Treg cells are present in the conjunctiva and can suppress herpes simplex virus (HSV)-1-specific CD4 and CD8 effector T cells. However, in the context of an acute viral infection, as it is the case of RHDV infection, the role of Treg cells is not so well studied and different infections models can have a different outcome. We show here that RHDV infection in adult rabbits is associated with a decrease frequency and number of CD4 Foxp3 in the spleen whereas no alteration in this cellular population is observed in young rabbits upon infection with the virus. In the MLN only a decrease in the frequency of CD4 Foxp3 is observed in adult rabbit upon infection and no alteration in the frequency of this cellular population is observed in the liver of infected young and adult rabbits comparatively to non-infected controls. Interestingly, it was shown by others that depletion of Tregs delays the arrival of populations of innate immunity to the site of viral infection. In a model of Con-A induced hepatitis it was shown that Treg cells protect against fulminant hepatitis. Therefore we can hypothesise that the decreased frequency of Treg cells observed in RHDV infected adult rabbits may affect the ability of the host to prevent fulminant virus-induced hepatitis. Further experiments will be needed to test this hypothesis and its putative mechanisms.

This work was funded by grants from FCT (PTDC/CVT/66656/2006), Portugal. Luzia Teixeira was supported by Fundo Social Europeu and MCTES through POPH-QREN- Tipologia 4.2.
UNDERSTANDING THE DUAL ROLE OF MURINE GAMMA-DELTA T CELLS IN TUMOR IMMUNE SURVEILLANCE

Telma Lança 1*, Margarida Rei 1,2*, Daniel J Pennington 2, Bruno Silva-Santos 1,3
1 Instituto de Medicina Molecular, Faculdade de Medicina de Lisboa; 2 Barts and The London, School of Medicine and Dentistry; 3 Instituto Gulbenkian Ciência, Oeiras, Portugal
*the authors contributed equally to this work

Abstract

γδ T cells have been widely shown to play non-redundant anti-tumor roles in cancer immunesurveillance. Their anti-tumor properties have been attributed to abundant IFN-γ production and strong cytotoxic properties; however, this view was recently questioned, when IL-17 production by γδ T cells was suggested to promote tumor growth. In order to unravel the cellular and molecular mechanisms behind this apparent paradox, we are using two different tumor models in which γδ T cells seem to play opposite roles. We observed that γδ T cells play a protective role against tumor progression following B16 melanoma cells transplantation, since tumors grow faster and bigger in TCRδ-deficient mice. By contrast, in the ID8 model of ovarian carcinoma, γδ T cells seem to play a pro-tumor role, as TCRδ-deficient mice survive longer than wild type mice. Interestingly, we observed a striking accumulation of IL-17-producing γδ T cells selectively in the ID8 model. By comparing these two models we expect to further dissect the functional properties of γδ T cells and to identify the molecular mediators of the anti- versus pro-tumor responses of particular γδ T cell subsets. We believe this will be important knowledge for the design of future γδ T cell-based cancer immunotherapy strategies.
Group B Streptococcus GAPDH is released upon cell lysis, associates with bacterial surface, and induces apoptosis in murine macrophages

Liliana Oliveira1,2,3,4, Pedro Madureira1,2, Elva Bonifácio Andrade1,2, Abdelouhab Bouaboud3,4, Eric Morello5, Paula Ferreira1,2, Claire Poyart3,4, Patrick Trieu-Cuot5 and Shaynoor Dramsi5

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5Institut Pasteur, Unité de Biologie des Bactéries Pathogènes à Gram-positif, CNRS URA 2172, 28 rue du Dr ROUX, 75724, Paris, France.

Abstract

Glyceraldehyde 3-phosphate dehydrogenases (GAPDH) are cytoplasmic glycolytic enzymes which, despite lacking identifiable secretion signals, have been detected at the surface of several prokaryotic and eukaryotic organisms where they exhibit non-glycolytic functions including adhesion to host components. Group B Streptococcus (GBS) is a human commensal bacterium that has the capacity to cause life-threatening meningitis and septicemia in newborns. Electron microscopy and fluorescence-activated cell sorter (FACS) analysis demonstrated the surface localization of GAPDH in GBS. By addressing the question of GAPDH export to the cell surface of GBS strain NEM316 and isogenic mutant derivatives of our collection, we found that impaired GAPDH presence in the surface and supernatant of GBS was associated with a lower level of bacterial lysis. We also found that following GBS lysis GAPDH can associate to the surface of many living bacteria. Finally, we provide evidence for a novel function of GAPDH as an inducer of apoptosis of murine macrophages.
Lympho-stromal interactions decline the frequency of IL-7-expressing thymic epithelial cells independently of the establishment of the medullary epithelium compartment.

Ana Rosalina Ribeiro1, Heidi Schmid1, Pedro Rodrigues1, Alexandra Moreira1, Alexandre do Carmo1, James P. Di Santo2, Nuno Lages Alves1

1 CellActivation and Gene Expression Group, Instituto de Biologia Molecular e Celular (IBMC), Porto, Portugal; 2 Innate Immunity Unit, Institut Pasteur, Paris, France

Abstract

The thymus provides a specialized microenvironment for the development of T cells. Thymopoiesis is not an exclusive thymocyte-intrinsic process and depends on unique migratory, survival, proliferative, commitment and selection signals provided by thymic epithelial cells (TECs). There are two known subsets of TECs, which differ in their function and spatial localization: cortical (cTECs) and medullary (mTEC). Both subtypes derive from a common precursor, undergoing a differentiation process that relies on reciprocal signals provided by thymocytes. However, we still know little about the molecular pathways responsible for TEC diversification. Interleukin 7 (IL-7) is an essential thymopoietic cytokine predominantly expressed by TECs. Using IL-7 reporter mice, in which yellow fluorescent protein (YFP) expression identifies TECs that co-express high levels of Il7 (Il7hi/YFP TECs), we have demonstrated that Il7hi/YFP TECs gradually decline during perinatal and adult life. Conversely, this subset is maintained in alymphoid mice Rag2(-/-)Il2rg(-/-) IL-7 reporter mice. Transfer of wild-type hematopoietic progenitors to alymphoid IL-7 reporter recipients normalizes the frequency of Il7hi/YFP TECs and re-establishes cortical TEC/medullary TEC segregation, suggesting that thymocyte-derived signals control the homeostasis of Il7hi/YFP TECs. Here, we study the lineage relationship between Il7hi/YFP TECs and the two-prototypical epithelial subsets throughout thymic organogenesis. We find that Il7hi/YFP TECs emerge early during thymic development, progressively acquiring, and retaining, a cTEC phenotype (CD205 BP1 CD40dim) and co-express cortical-associated thymopoietic factors (Dll4, Ccl25, Psmb11(b5t)), segregating from CD80 CD40highAire mTECs. The establishment of mature mTECs depends the TNF receptor superfamily RANK ligand provided by developing thymocytes. Using fetal thymic organ cultures, we show that the RANK-induced formation of Aire mTECs and the thymocyte-mediated decrease in Il7hi/YFP TECs are two independent events. Together, our results indicate that Il7hi/YFP TECs are a determinant of a cortical epithelial lineage and that distinct thymocyte-derived signals controlled the establishment of the cortical and medullary compartment.
The role of B1 cells in the early steps of type 1 diabetes

Nadia Duarte, Joana Côrte-Real, Carlos Penha-Goncalves
Instituto Gulbenkian de Ciência, Oeiras, Portugal

Abstract

Background and aims: Type 1 diabetes (T1D) is generally known as a T-cell mediated autoimmune disease where the pancreatic beta-cells are destroyed and insulin secretion abrogated with severe metabolic consequences. Yet, B lymphocytes have been proven necessary for pathogenesis and detection of autoantibodies to beta-cell antigens is one of the earliest indicators of disease. In particular, insulin autoAbs have predictive value both in human patients and in the non-obese-diabetic (NOD) mouse, a widely used animal model that spontaneously develops T1D in a similar manner to the human condition. Nevertheless, the origin and the role of beta-cell specific autoAbs in T1D development remain obscure. Unraveling the root of autoAbs generation and identifying linked B cell dysfunctions leading to disease will allow optimal diagnosis and early therapeutic interventions in T1D. B1 lymphocytes constitute a distinct B cell population of fetal origin, characterized by the expression of CD5 molecule and secretion of Natural antibodies (NAbs). We put forward the unexplored hypothesis that B1 cells and the natural autoantibodies they produce, are involved in T1D early pathogenesis.

Materials and methods: We have analyzed the phenotype and function of B1 cells in several organs of the NOD mouse in comparison to the C57BL/6 control mouse strain. Thus we have characterized by flow cytometry the distribution of B1 cells in several organs as well as the expression of molecules on their surface. Also, we have isolated B1 cells and determined by ELISPOT their ex vivo ability to secrete immunoglobulins recognizing T1D related autoantigens. In addition we have characterize by Real time PCR the expression of TLR receptors and performed in vitro culture of purified B1 cells with and without agonist to TLR4 (LPS) to characterize their response to innate stimuli. ELISA and ELISPOT were used to analyze the secretion pattern of IL10 and Immunoglobulins and thimidine incorporation was performed to determine proliferation in these cell cultures. The effect of NOD IgM produced by B1a cells on NOD.SCID beta cells was examined in transwell co-cultures. IgM binding was measured by flow cytometry and Real-Time PCR used to study oxidative stress responses.

Results: We have observed that that B1 cells from the peritoneal cavity are the main secretors of IgM recognizing T1D related autoantigens. Further, young NOD mice without pancreatic infiltration present a B1 cell repertoire with increased self-reactive in comparison to B6 mice. Also NOD B1 cells show a higher expression of activation markers prior to pathogenesis onset indicating an increased basal level of activation in these cells. Interestingly, the levels of several innate Toll-like receptors (TLR) are increased in NOD B1 cells and in vitro stimulation with a TLR4 agonist resulted in the secretion of higher amounts of antibodies recognizing pancreatic antigens and less IL10 secretion by NOD B1 cells in comparison to the control mouse strain. In addition, spontaneously secreted IgM of NOD B1a cells origin was able to bind to pancreatic beta cells in vitro and induce iNOS expression in islet cultures.

Conclusions: NOD B1a cells have an increased responsiveness to innate activation and secrete natural antibodies with higher reactivity to T1D associated AAg. Importantly, NOD B1a cell-derived IgM is able to bind pancreatic beta cells and may trigger iNOS expression, a starting point in the beta cell oxidative stress response. In conclusion, we have linked alterations in the B1a cell population to serum IgM autoactivities and beta cell oxidative stress strengthening the hypothesis that NAbs are an early factor in T1D pathogenesis evolving in the NOD mouse.
The fate of CD4 T cells under tolerogenic stimulation: a modeling perspective

M. Caridade\textsuperscript{1}, V. Oliveira\textsuperscript{1}, A. Agua-Doce\textsuperscript{1}, R.M. Ribeiro\textsuperscript{2}, L. Graca\textsuperscript{1}

\textsuperscript{1}Instituto de Medicina Molecular, University of Lisbon, Lisbon, Portugal; \textsuperscript{2}Theoretical Biology and Biophysics, Los Alamos National Laboratory, Los Alamos, NM, USA.

Abstract

Immunological tolerance can be defined as the state of unresponsiveness to an antigen, following prior contact with that antigen, where the host remains competent to mount an effective immune response against third-party antigens. Accomplishing therapeutic induced tolerance has been one of the major goals of immunology.

Several monoclonal antibodies (MAbs) have been used to induce tolerance. One such MAbs, non-depleting anti-CD4, was shown efficient in inducing long-term tolerance in several models of transplantation and autoimmunity.

Tolerance induced using non-depleting anti-CD4 MAb is considered robust, as a state of dominant tolerance, that can resist the adoptive transfer of large numbers of cells with the potential to mediate graft rejection, is achieved. Despite many studies on tolerance induction using non-depleting anti-CD4 MAb, the role played by this molecule on T cell dynamics still remains to be elucidated. Some reports have pointed out that the role of non-depleting anti-CD4 MAb in tolerance induction can be related to apoptosis of activated antigen-specific T cells.

In recent years mathematical models have been used to understand lymphocyte dynamics, estimating rates of the first and subsequent divisions, and death during proliferation. These studies have focused mainly on the role of key cytokines on T and B lymphocytes activation under a potent stimulus. The parameters mentioned above were measured in a quantitative manner and modeled accordingly. Using the same strategy it is possible to depict the effect that non-depleting anti-CD4 MAb has on T cell dynamics shedding some light on the processes of tolerance induction.

Our in vitro studies of T cell fate after activation under the tolerogenic effect of non-depleting anti-CD4 seem to show that the non-depleting anti-CD4 MAb does not lead to apoptosis of T cells, but rather impairs the the proportion of cells that enter the division.
BAFF-R and TACI expression in Common Variable Immunodeficiency – insights into receptor dynamics

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Abstract

Common Variable Immunodeficiency Disorders (CVID) are defined by impaired antibody production due to defective mature B-cell function. The interaction of the B-cell activating factor receptor (BAFF-R) and transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) with their ligands, BAFF and a proliferation-inducing ligand (APRIL), is crucial for peripheral B-cell survival and differentiation. Thus, alterations in these pathways are likely to contribute to CVID. We evaluated the expression of BAFF-R and TACI in a cohort of 31 CVID patients and age-matched healthy individuals, and studied its relationship with BAFF and APRIL serum levels. Although CVID is fundamentally considered to be of polygenic etiology, monogenic defects affecting these receptors have been described in some cases, and mutations in BAFF-R and TACI were therefore excluded in these patients. BAFF-R only binds BAFF, whereas TACI can bind both BAFF and APRIL. The serum levels of both BAFF and APRIL were markedly increased in CVID patients. BAFF-R expression was uniformly reduced on B cells from CVID patients and it was inversely correlated with BAFF levels, both in CVID patients and healthy controls. TACI expression was significantly increased on B cells from CVID patients and its expression on memory B cells was directly associated with BAFF levels. Of note, this was not observed for APRIL. These results suggest that BAFF-R expression may be regulated by ligand binding. We have been testing this hypothesis in vitro and the preliminary results suggest that BAFF-R expression is down-regulated upon BAFF binding, possibly through internalization. Ongoing and future studies regarding this dynamics, both in healthy subjects and CVID patients, will be important to understand the role of BAFF-R and TACI on CVID pathogenesis, in particular, and B-cell biology, in general.
Intravital imaging acquisition of uterus: the mystery of DC distribution before implantation

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Abstract

Pregnancy can be considered as a challenge to the maternal immune system as it has to maintain the body defenses against pathogens while developing immune tolerance against the semiallogeneic fetus growing inside the uterus. The uterus has maternal immune cells and the fetal tissue is in direct contact with maternal blood containing these cells with the potential of destroying structures bearing foreign antigens. However, it is known that fetal structures are actively tolerated rather than ignored. By the development of a new "stereotactic" holder, surgical procedures, and use of a two-photon microscope, we present methods to allow in vivo observation of dendritic cells (DCs) inside the mouse uterus. Using these methods we could observe DC distribution inside the uterus before pregnancy, at different phases of estrus cycle. The results show a differential distribution of DCs and this could be related to previous modifications to accept implantation.
Molecular mechanisms of differentiation of pro-inflammatory gamma-delta T cell subsets

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Abstract

The cytokines Interferon-gamma (IFN-g) and Interleukin-17 (IL-17) are critical mediators of the pro-inflammatory activity of T cells in contexts of infection, cancer and autoimmunity. Thus, the study of the regulation of Ifng and Il17 gene expression is of major biological and clinical relevance. Our laboratory has recently shown that gamma-delta (gd) T cells contain two distinct subsets, segregated on the basis of CD27 expression, that produce large amounts of either IFN-g or IL-17 (Ribot et al. Nat Immunol 2009). However, the molecular mechanisms that underlie Ifng and Il17 gene expression in gd T cell subsets remain poorly understood.

We first aim at characterizing the epigenetic marks that regulate Ifng and Il17 expression in gd T cells, for which we have performed chromatin immunoprecipitation followed by deep sequencing (ChiP-Seq) experiments on the distinct functional subsets. These provided a full map of the histone modification marks, allowing the establishment of epigenetic signatures that segregate with each of the gd T cell subsets. Secondly, we aim at dissecting the role of microRNA (miRNA) in the post-transcriptional regulation of Ifng and Il17 expression and to functionally characterise novel miRNA involved in Ifng or Il17 regulation. For this, we have undertaken a transcriptome-wide (microarray) analysis of miRNA expression in the IFN-g (CD27+) and IL-17 (CD27-) gd T cell subsets. We are currently validating these data and initiating the functional characterization of the most promising miRNA candidates.

In summary, our work constitutes a comprehensive study of key molecular events that underlie gd T cell differentiation in vivo, addressing pre- and post-transcriptional mechanisms that initiate and maintain such programmes in distinct gd T cell subsets. The results obtained will advance our understanding of the functional differentiation of gd T cells, thus contributing for translational research in the areas of infection, cancer and autoimmune diseases.
Skin-regulatory T cell disturbance after Plasmodium berghei sporozoite infection

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Abstract

Although the role of regulatory T cells (Tregs) during malaria infection has been studied extensively, both in mouse models and humans, such studies have focused exclusively on the role of Treg during the blood stage of infection. Importantly, while nothing is known about detailed mechanisms of Tregs and sporozoite deposition in the dermis by mosquito bite, it is clear that the skin serves as a major site for the immunosuppressive action of Tregs. In this paper we show that sporozoites introduced in the skin by mosquito bites increase the mobility of skin Tregs, thereby leading to the suppression of MHC class II and/or CD86 on antigen presenting cells, including both dendritic cell subtypes and macrophages. Thus, our data suggest that Tregs may start to play a role during malaria infection even in the early phases of parasite inoculation into the mammalian host.
CD70 on dendritic and epithelial cells in the thymic medulla promotes CD4 Foxp3 regulatory T cell development via CD27

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Abstract

CD4 Foxp3 regulatory T cells (Treg) are largely self-reactive, yet escape clonal deletion in the thymus. We demonstrate here that CD27/CD70 costimulation rescues thymic Treg precursors from apoptosis and promotes Treg development. Genetic ablation of CD27 or its ligand CD70 did not affect the development of conventional CD4 Foxp3- T cells, but significantly reduced Treg numbers in the thymus and periphery. CD27 was not required for Foxp3 induction, the functional programming of Treg or their proliferation. Rather, CD27 enhanced the positive selection of Treg within the thymus, in a cell-intrinsic manner. CD27 limited pro-apoptotic gene expression in CD4 CD25 Treg precursors and promoted their survival, while having no apparent effect on CD4 CD25- T-cell precursors. CD70 was found in the thymic medulla, on epithelial cells and conventional dendritic cells (cDC). In vitro, we specified that CD70 on CD8a cDC supported Treg development. Using newly generated CD70-deficient mice, we established that CD70 on both DC and epithelial cells contributed to Treg development in vivo. These data emphasize that Treg development in the thymic medulla has different costimulatory requirements than conventional CD4 T cell development and identify the CD27/CD70 costimulatory system as an important determinant of the size of the Treg population under homeostatic conditions.
Cytokine expression during RHD: differences between adult and young rabbits

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Abstract

Rabbit Haemorrhagic Disease (RHD) is a lethal viral infection that kills 40-90% of adult rabbits of the species, Oryctolagus cuniculus, within 3 days. RHD causes fulminant hepatitis associated with disseminated intravascular coagulation. Young rabbits, however, are natural resistant to RHD, developing only transient subclinical hepatitis. Our previous data on the pathogenesis of RHD allow us to postulate that differences in the inflammatory response of young and adult rabbits may contribute to the different outcome of the RHDV infection in young and adult rabbits. In the present work we studied the kinetics of several cytokines (TNF-α, IL-1, IFN-α, IFN-γ, IL-8, IL-6 and IL-10) in infected young and adult rabbits. Young rabbits were studied up to 7 days after infection whereas adult rabbits were followed until death that occurred within 24-36 hours of infection. TNF-α analysis of serum of infected adult rabbits showed an early and exponential increase during the infection, confirming the recent report by Tunon and coworkers (2010). They considered that overexpression of TNF-α, in the liver of infected adult rabbits may cause the massive liver cell apoptosis of RHD. In contrast, young rabbits showed only a substantial increase in TNF-α during the acute phase (24-48 hours). IFN-α and IL-1 values of infected young rabbits were gradually increased until 18 hours after infection; adult rabbits presented an increase, but significantly inferior to that registered in young rabbits. IL-6 analysis, in the serum of infected young rabbits, showed two peaks: during acute phase (24-48 hours) and 7 days after viral inoculation; this suggests that IL-6 may have an important role in both inflammatory response and liver regeneration. IL-8 presented a mild increase at 18 hours after infection and it was present 7 days later. In conclusion, we propose that differences observed in inflammatory response between the two age groups of rabbits may contribute to the different outcome of the viral infection, namely, RHDV infection, in adult rabbits, induced a "cytokine storm" that led to severe liver damage and death by fulminant liver failure; in young rabbits, in contrast, inflammation was associated with an effective anti-viral response against RHDV infection.

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Serum Immunoglobulin (Ig) titer is maintained by plasma cells (PC) that develop spontaneously in steady state or upon antigen encounter. Plasmablasts (PB), precursors of post-mitotic PC, are generated in extrafollicular foci or in germinal centres (GC) of secondary lymphoid organs. Mature PC persist in confined foci in the red pulp of the spleen or in the bone marrow, where they compete for survival factors including APRIL. AID-/ µS-- mice lack both AID, the enzyme promoting the formation of secreted Ig isotypes through class switch recombination, and µS, the DNA motif encoding the secretory signal of the IgM. In these mice devoid of secreted Ig, we assessed whether secretion of Ig is a requirement for PC differentiation in steady states. We found that the spleens of AID-/ µS-- mice compared to those of wild type mice contained higher numbers of activated B cells expressing IRF-4, the transcription factor initiating PB differentiation, as well as more BCL-6 GC B cells. Analyses by real-time PCR and immunohistology showed that transcripts for IRF-4 and Blimp-1 - another transcription factor necessary for the terminal differentiation of PC - are over-expressed in AID-/ µS-- spleens. Moreover, the extra PB mislocalize in the follicles and marginal zones, fail to reach the red pulp and do not up-regulate CD138, a receptor for APRIL involved in their maturation and survival. These data demonstrate that Ig secretion is required for the final differentiation of PC and their accumulation in splenic long-term survival microenvironments, and suggests that levels of serum Ig help control the numbers of B cells entering PC differentiation programme in steady states.
Vβ Repertoire at the TCRβ locus: Contribution of the Recombination Signal Sequence and the Epigenetic Marks to the Recombination Efficiency

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Abstract

The generation of a very large repertoire of T and B cell antigen receptors in vertebrates relies on a mechanism of somatic DNA recombination. The lymphocyte specific Recombinase Activating Genes (RAGs) introduce DNA breaks at specific sequences -named Recombination Signal Sequences (RSS)- which border antigen receptor gene segments. Many lymphoid tumours originate from mistargeted RAG activity to RSS-like sequences (cryptic RSS) bordering proto-oncogenes. A rigorous method to predict the recombination potential of a given DNA sequence in its specific chromatin structure would serve to formally establish the frequency and nature of cryptic RSS in mammalian genomes. This will further enable the development of prognostic tools for leukaemia.

In a first attempt to better describe the recombination potential of a given sequence, we use the T Cell Receptor beta (TCRb) locus as a model and more specifically we study the usage of Vbeta gene segments (Vb) in the repertoire of early T cells. We first aim to test all Vb-RSS in an in vitro assay for their recombination efficiency in an epigenetic-mark free system, using a novel fluorescent-based readout. In parallel, we will determine the exact early repertoire of the Vb gene segments by using a specific array to capture rearranged TCRb DNA from early T cells, and by high-throughput sequencing of the recovered DNA. Also, we will define epigenetic patterns of the Vb-RSS and their potential accessibility to the recombination machinery, using ChIP-on-chip and chip-Seq data from early T cells.

Finally, we aim to quantify the relative importance of DNA accessibility compared to the RSS nucleotide sequences for the effectiveness of rearrangements, using mathematical modelling.

By using the TCRb locus as a model and by combining expertise on VDJ recombination, T-cell repertoire, epigenetics and mathematical modelling, this project aims to accurately define RAG targeting rules.

Future work will use the same approach but incorporating all-genome bio-informatic analysis to systematically predict candidate sequences that might be involved in translocation and deletion events.
Peripheral administration of foreign antigens promotes a dose dependent intrathymic selection of specific regulatory T cells.

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Abstract

Thymocytes differentiate into CD4 Foxp3 regulatory T cells (Treg) upon interaction between their TCR and peptide-MHC-II complexes expressed in the thymus by either Dendritic Cells (DC) or by Thymic Epithelial Cells (TEC). We have recently established that antigen (Ag) specific Treg are de novo generated in the thymus in response to pro-inflammatory peripheral immunization, indicating that peripheral antigens access the thymus and locally promote Treg differentiation. Here, we investigated the conditions favoring the selection of intra-thymic T cells reactive to foreign antigens administrated in the periphery (True Peripheral Antigens - TPAg), both in inflammatory and non-inflammatory conditions. By analyzing transgenic mouse strains monoclonal for TCRs specific for either Ovalbumin (OVA) (on two different genetic backgrounds) or for the male HY Ag, we evidenced that intra-thymic differentiation of Treg specific for TPAg occurs prior to peripheral Treg detection and is independent: i) of adjuvants, ii) of the route of antigen delivery (Foot-Pad, intra-venous or oral administration) and iii) of the Ag molecular weight (peptide or whole protein). The dose of the peripheral Ag shapes intra-thymic selection events including T cell expansion, Treg induction and deletion, such that for each TCR-Ag pair, maximal Treg differentiation occurs at a specific Ag dose. Finally, we confirm that thymic DC efficiently presents proteins administrated in the periphery -even by the oral route. This phenomenon has major implications for the understanding of how CD4 T cell repertoires are determined, since it reveals that the antigens presented to developing T cells are not restricted to intra-thymically synthesized proteins and small blood born peptides, as it has been for long assumed. Moreover, our finding that antigens administered in the periphery participate in intra-thymic T cell selection has major implications for the design of corrective peptide and whole protein therapies for autoimmune disease.
Abstract

Under conditions associated with oxidative stress, hemoglobin can release its prosthetic group, generating "free heme", which acts in a cytotoxic manner, probably explaining why it appears to act as a central component in the pathogenesis of several immune mediated inflammatory diseases (1-4). There are currently no analytical methods that allow to discriminate and quantify free heme or that provide informations on its molecular environment after its release from hemoproteins. To overcome this limitation we are developing analytical approaches to characterize free heme biologically. For that purpose, we are trying to produce antibodies specific against free heme to be used in an enzyme linked immunoabsorbant assay (ELISA) in order to be able to quantify it and in other possible methods. We synthesized and purified biotinylated heme to be used in phage display technology (5). We are in the process to select antibodies with high affinity and specificity for heme, which will be produced in high scale and screened in order to select the most efficient. The selected peptides will be purified and their affinities for free heme will be tested by ELISA. Moreover, we will assess in vitro and in vivo if the obtained antibodies could modulate the cytotoxic effect of free heme.

References:
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Abstract

Foxp3 CD4 regulatory T cells (Treg) are key players in the regulation of immunological tolerance. While natural Treg develop in the thymus from newly formed thymocytes, peripheral CD4 Foxp3⁺ T cells may acquire Foxp3 expression upon stimulation in non-inflammatory conditions. In this work we directly assess the influence of maturation stage on T cell susceptibility to acquire Foxp3 expression outside the thymus.

To this aim we made use of the model of lymphopenia induced proliferation that associates with de novo Foxp3 cell differentiation. We first evidence that CD4 Foxp3⁻ SP thymocytes are more prone than LN CD4 Foxp3⁻ cells to differentiate into Foxp3 Treg upon adoptive transfer into T cell deficient animals. We then established that this property is a cell intrinsic feature, does not require the thymus and is dependent on peripheral antigen encounter. Next, by performing thymectomies on the donor animals, or by fractioning peripheral CD4 Foxp3⁻ cells according to their maturation stage, we further reveal that recent thymic emigrants (RTE) are the major source of Treg differentiated in the periphery upon lymphopenia induced proliferation. Finally, by performing in vitro assays, we established that RTE, like thymocytes, display lower sensitivity than peripheral resident mature cells to the inhibitory effects of IL6 and IL4 on the differentiation of Treg.

Our findings, indicating that T cell maturation stage conditions de novo Foxp3 expression and that RTE are the preferential precursors of Treg differentiated in the periphery, provide both experimental evidence and a mechanism in support of early models of self-tolerance establishment. According to these models, immature cells would be bound to tolerizing differentiation pathways, for which Foxp3 Treg differentiation is now a days the perfect candidate. These findings, once confirmed in humans, should help guiding the design of Treg based therapies. Notably, given that thymic output decreases with age, it indicates that tolerogenic peptide regimens would be more efficient if administered to young individuals.
Lymphoid tissue inducer (LTi)-like cells in human tonsils and colon mucosa

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Abstract

Lymphoid tissue inducer (LTi)-like cells are closely related to fetal LTi cells described during lymphoid organ morphogenesis. They belong to a large family of innate lymphoid cells and were recently shown to play a pivotal role in the homeostasis of adult secondary lymphoid organs (SLO). Murine studies have shown that LTi-like cells are critical for tissue remodeling and repair upon injury, which might be particularly important in the context of persistent infections like HIV. Human LTi-like cells are defined by the expression of cKit, NKp44, IL-7Rα and absence of lymphoid lineage markers. It is also believed that their development depends on Id2 and RORγt. Finally, LTi –like cells were shown to produce IL-22 and IL-17 and were therefore named ILC22 and ILC17, respectively. Herein we compare the phenotype and function of LTi-like cells obtained from human inflamed tonsils and colon biopsies.

Tonsils were collected during routine tonsillectomies performed mainly due to tonsil hyperplasia, and immediately processed. After mechanical tissue dispersion, lymphocytes were separated on a Ficoll-Paque® gradient. In parallel, explants tonsil cultures were performed, and analyzed at day 0, 2 and 8. Six to 10 colon biopsies, collected from healthy individuals during colonoscopy for colorectal cancer screening, were digested with collagenase and lymphocytes separated on a Percoll® gradient. Cytokine production was assessed following short-term culture with PMA and ionomycin. Analysis of cell suspensions by 8-color flow cytometry was combined with immunohistochemistry studies in paraffin and immunofluorescence studies in frozen tissue.

We found that comparatively to their colon counterparts, tonsil LTi-like cells had lower levels of cKit and IL-22 expression. Importantly, IL-22 production was seemingly dissociated from IL-17 production both in gut and tonsils. Moreover, despite increasing data suggesting the possibility that these cells produce IFN-γ upon inflammatory signals, no significant IFN-γ-production was found in either tissue. Interestingly, LTi-like cells cultured in explants tonsil cultures up-regulated cKit to levels similar to those found in the colon. Altogether, our results suggest that the tonsil inflammatory milieu favors the emergence of ckitlow in detriment to cKithigh, and that the acquisition of a ckitlow phenotype might be reversible as indicated by cKit up-regulation upon explants culture. In this context, we are currently investigating the role of LTi-like cells during persistent HIV infection and will study whether they are involved in the disruption of SLO homeostasis thought to underlay AIDS progression.

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HIV-2 has a reduced ability to infect the human thymus.

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Abstract

HIV-2-associated immunodeficiency has a limited impact on the survival of the majority of infected adults and is thus considered a unique natural model of attenuated HIV infection. Our previous data suggest that HIV-2-infected individuals have preserved thymopoiesis, as estimated by sj/βTREC (TCR excision circles) ratio, which may contribute to the slow rate of CD4 decline that characterizes HIV-2 infection through a continuous replenishment of the peripheral naïve T cell pool.

Here we investigated the direct impact of HIV-2 infection on the human thymus in vitro. Assays using thymocytes from human thymic tissue, removed according to standard current practice from children undergoing reconstructive cardiac surgery, were used to study the ability of HIV-2 to infect and replicate in different subsets. We compared HIV-2 or HIV-1 primary isolates using either CXCR4 or CCR5 co-receptor. CXCR4 is widely expressed in human thymocytes, whereas CCR5 is found at very low levels in mature T cells.

We found that both the type of virus and co-receptor used contributed in defining the profile of viral integration (proviral DNA levels), viral replication per cell (frequency of cells expressing intracellular Gag viral protein) and cumulative amount of viral production (viral reverse transcriptase activity in supernatants).

Infection of whole thymocytes by HIV-2 was associated with much lower levels of intracellular Gag viral protein than HIV-1, irrespective of co-receptor use. Importantly, infection of purified CD4SP thymocytes by HIV-2 resulted in lack of detectable intracellular Gag protein. Nevertheless, HIV-2 was able to enter and subsequently integrate into the thymocyte genome, as significant levels of proviral DNA were observed.

Of note, both HIV-1 and HIV-2 were able to infect regulatory T cells (Treg), identified by the expression of Foxp3. In addition, CXCR4-tropic HIV-1 was associated with a preferential depletion of Foxp3 cells in culture.

In conclusion, we provide here, for the first time, evidence of an impaired ability of HIV-2 to replicate in human thymocytes, particularly mature CD4SP cells, supporting a differential impact of HIV-2 and HIV-1 infection on the human thymus.
Infected but not Bystander Dendritic Cells polarize CD4 T cells towards a non-protective T-bet INF-γ IL10 phenotype

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Abstract

The knowledge of the complex interactions between *Leishmania* and dendritic cells (DCs) is central to potentially modulate the outcome of this infection given that an effective immune response against these trypanosomatid parasites is dependent on a successful activation/maturation of DCs. The differential analysis of infected and bystander DC-populations using CFSE labeled-*L. infantum* parasites clearly indicated the presence of two types of responding cells: while bystander DCs showed an up-regulated profile, the infected cells exhibited a more immature state. A similar profile was observed after 24 hours of in vivo infection. Interestingly, we demonstrate that Leishmania actively downregulated surface MHCII on mature DCs, which was specific to the infected population. In addition, bystander DCs were found to upregulate IL-6 and IL-12p40 production, whereas TNF-α was preferably detected on the infected population. In vitro co-cultures demonstrated that infected DCs were unable to induce CD4 T cell activation in opposition to bystander DCs, in a process dependent on IL-10 secreted by DCs. Whereas the latter induced preferentially an INF-γ producer Th1 lymphocytes, the infected DCs polarized CD4 T cells into a T-bet INF-γ IL10 profile in a cell-contact mediated process. *Ex-vivo* splenic bystander and infected DCs also induced a similar behavior on CD4 T cell differentiation. Adoptive transfer of CD4 T cells previously primed by infected DCs into infected Balb/c mice clearly demonstrated the incapacity of the T-bet INF-γ IL10 cells to mount an effective immune response. Overall, we have identified a mechanism by which non-protective T-bet INF-γ IL10 T cells are developed which is associated with chronicity and responsible for prolonged persistence of parasite.
Notch signaling enhances the in vitro differentiation of human Regulatory T cells

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Abstract

Regulatory T cells (Treg) that constitutively express the transcription factor FOXP3 are fundamental to prevent autoimmunity. This is directly supported by the observation that Foxp3 null mutations, in both humans and mice, lead to early and fatal spontaneous autoimmunity. Similarly, diminished frequency and/or dysfunction of Treg have been reported in several human and murine autoimmune diseases. Adoptive transfer of Treg has proved to be highly efficient both in the prevention and treatment of autoimmunity in rodents. However, these studies established the need for large numbers of Treg to achieve therapeutic efficacy, especially in animals with established autoimmunity. Since in humans the number of Treg that can be isolated from the periphery are far too small for adoptive therapy, the establishment of novel protocols leading to their competent ex vivo expansion and/or efficient and stable conversion is vital. The Notch signaling pathway is known to be important at several stages of T cell development and differentiation. In mice, Notch signaling enhances the thymic generation of Treg, their peripheral expansion and the in vitro conversion of conventional T cells into the Treg phenotype. The putative role Notch may play in human Treg differentiation has never been thoroughly investigated. Our preliminary data show that pharmacologic inhibition of Notch significantly reduces the proportion of cells acquiring Foxp3 in standard ex vivo conversion protocols, using both sort-purified non-regulatory thymocytes and peripheral naive or memory CD4 T cells. We have also co-cultured each of the above-described sort-purified populations with OP9 cells expressing the Delta-like 1 (DL1) ligand and control OP9 stroma cells, in conditions favoring the induction of FOXP3. We observed that DL1 significantly enhanced the differentiation of conventional cells into Foxp3-expressing cells. These induced Treg expressed higher levels of FOXP3 as well as other Treg-associated markers as compared with Foxp3-expressing cells differentiated in a co-culture system using control stroma cells. The results obtained with both strategies suggest that Notch enhances the induction of FOXP3 on non-regulatory cells, supporting a role, particularly for DL1, in human Treg differentiation. Manipulation of this signaling pathway may therefore help the use of Treg-based therapies for the treatment of autoimmunity.
HIV unveils: regulated vesicle fusion conveys T cell receptor signaling

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Abstract

CD4 T cells are crucial in protecting against infections, tumors and in controlling autoimmunity. T cells systematically scan for non-self antigens presented within major histocompatibility complex molecules on the surface of antigen presenting cells. Antigen recognition generates a specialized interface between the T cell and the antigen presenting cell named immunological synapse where T cell receptor (TCR) signaling is sustained and controlled. Intracellular vesicle transport targets the TCR, the tyrosine kinase Lck and the adaptor LAT to the synapse, but how this transport is regulated and its significance for signal integration remain unknown. By studying HIV-1 modulation of the vesicular traffic conducive to immunological synapse formation, we disclosed a novel mechanism for TCR signal integration controlled by hierarchic recruitment of vesicle-associated signaling molecules. It requires at its inception Lck delivery, which in turn induces the fusion of TCRζ and LAT from subsynaptic vesicles. This tightly regulated vesicle fusion cascade builds up the immunological synapse signaling breadth and its ultimate capacity to prime IL2 and IFNγ production. Such mechanism may contribute to elucidate how signal compartmentalization is translated into different T cell activation outcomes and how its subversion by HIV infection modulates HIV-specific immune response and overall disease progression.
Activation of IL-27 p28 gene transcription on antigen presenting cells infected with *Leishmania infantum*

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**Abstract**

*Leishmania* parasites are obligate intracellular eukaryotic protozoan pathogens that thrive on the mononuclear phagocytic system. The virulence related to the pathology seems to be linked to an induced lack of immune response control that begins with the manipulation of innate immune cells. In this context, cytokines are critical coordinators of the immune response necessary for resolving the infection. In particular, the IL-12 family of cytokines are key players in the initiation and regulation of cell-mediated immunity. The bioactive IL-12p70 is critical for the induction of Th1 responses. Other two members of this family, IL-23 and IL-27 have been demonstrated to induce or antagonize, respectively, the development of inflammatory Th17 cells. Bioactive IL-27 composed by p28 and Ebstein-Barr-Virus-induced gene 3 (EBI3) has been similarly associated with the initiation of Th1-type immune responses and the attenuation of immune/inflammatory responses in various experimental settings. In this study, we have evaluated the kinetics of mRNA production of each member of the IL-12 family (p19, p28, p35, p40, EBI-3) in bone marrow macrophage (BMM) and dendritic cells (BMDC) infected *in vitro* with *L. infantum* promastigotes. A general downregulation of all these members was observed with the exception of IL-27p28 subunit, which was found upregulated. In agreement, increased levels of p28 secretion but not bioactive IL-27 were found in the supernatant of infected BMM and BMDC. Moreover the expression of IL-27p28, in opposition to IL-12p35 and IL-12p40, was found to be independent of IL-10. More importantly, macrophage and dendritic cells recovered from the spleen of acutely infected mice correlate the in vitro results demonstrating a specific increase of IL-27p28 subunit. Taken together, these results suggest a potential role for IL-27p28 subunit, which can exert biological activities independently of EBI3, in the modulation of early events of infection that will warrant further studies.
Differentiation of human peripheral blood Vδ1 T cells expressing the natural cytotoxicity receptor NKp30 for recognition of lymphoid leukemia cells.

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Abstract

The success of cancer immunotherapy depends on productive tumor cell recognition by killer lymphocytes. γδ T cells are a population of innate-like lymphocytes endowed with strong, MHC-unrestricted cytotoxicity against tumor cells. This notwithstanding, we recently showed that a large proportion of human hematologic tumors is resistant to γδ peripheral blood lymphocytes (PBLs) activated with specific agonists to the highly prevalent Vy9Vδ2 TCR. Although this probably constitutes an important limitation to current γδ T cell-mediated immunotherapy strategies, we describe here the differentiation of a novel subset of Vδ2(-) Vδ1( ) PBLs expressing natural cytotoxicity receptors (NCRs) that directly mediate killing of leukemia cell lines and chronic lymphocytic leukemia patient neoplastic cells. We show that Vδ1( ) T cells can be selectively induced to express NKp30, NKp44 and NKp46, through a process that requires functional phosphatidylinositol 3-kinase (PI-3K)/AKT signaling on stimulation with γ(c) cytokines and TCR agonists. The stable expression of NCRs is associated with high levels of granzyme B and enhanced cytotoxicity against lymphoid leukemia cells. Specific gain-of-function and loss-of-function experiments demonstrated that NKp30 makes the most important contribution to TCR-independent leukemia cell recognition. Thus, NKp30( ) Vδ1( ) T cells constitute a novel, inducible and specialized killer lymphocyte population with high potential for immunotherapy of human cancer.
Differentiation of human peripheral blood Vδ1 T cells expressing the natural cytotoxicity receptor NKp30 for recognition of lymphoid leukemia cells

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Abstract

The success of cancer immunotherapy depends on productive tumor cell recognition by killer lymphocytes. γδ T-cells are a population of innate-like lymphocytes endowed with strong, MHC-unrestricted cytotoxicity against tumor cells. This notwithstanding, we recently showed that a large proportion of human hematological tumors is resistant to γδ peripheral blood lymphocytes (PBLs) activated with specific agonists to the highly prevalent Vγ9Vδ2 T-cell receptor (TCR). While this likely constitutes an important limitation to current γδ-T-cell-mediated immunotherapy strategies, we describe here the differentiation of a novel subset of Vδ2(-) Vδ1 PBLs expressing natural cytotoxicity receptors (NCRs) that directly mediate killing of leukemia cell lines and chronic lymphocytic leukemia patient neoplastic cells. We show that Vδ1 T-cells can be selectively induced to express NKp30, NKp44 and NKp46, through a process that requires functional PI-3K/ AKT signaling upon stimulation with gc cytokines and TCR agonists. The stable expression of NCRs is associated with high levels of Granzyme B and enhanced cytotoxicity against lymphoid leukemia cells. Specific gain-of-function and loss-of-function experiments demonstrated that NKp30 makes the most important contribution to TCR-independent leukemia cell recognition. Thus, NKp30 Vδ1 T-cells constitute a novel, inducible and specialized killer lymphocyte population with high potential for immunotherapy of human cancer.
Plasmin(ogen) acquisition at the surface of Group B Streptococcus promotes the invasion of the Central Nervous System

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Abstract

Group B Streptococcus (GBS) is the leading cause of meningitis in newborns. We have previously shown that the binding of GBS to human plasmin(ogen) lead to the acquisition of proteolytic activity by the bacterium. Here we investigated whether plasmin(ogen) coated GBS enhances the bacterial invasion of human brain microvascular endothelial cells (HBMEC), the main component of the blood-brain barrier. For that purpose, HBMEC monolayers were infected with the serotype III GBS NEM316 pre-coated or not with plasmin(ogen), and the adhesion and invasion capacity were assessed. The bacteria coated with plasmin(ogen) lead to a four fold increase in the HBMEC invasion and adhesion, comparatively with the uncoated GBS, 90 min post-infection. To evaluate the direct cytotoxic effects of the surface-bound plasmin(ogen) GBS, the HBMEC viability was assessed by the Neutral Red assay. A higher loss of HBMEC viability was observed in cells infected with plasminogen coated GBS, which is in accordance with the increased ability of GBS to pass through the HBMEC monolayer when coated with plasmin(ogen). To further confirm our hypothesis that the subversion of the host plasminogen system by GBS contribute to the pathogenesis of bacterial central nervous system infection, neonate mice were intraperitoneally infected with 106 cells of either GBS or plasmin(ogen)-coated GBS. As expected, the brain bacterial load of neonates mice challenged with plasmin(ogen) coated GBS cells was significantly higher than in pups challenged with uncoated GBS.

In conclusion, these results indicate that the plasminogen system may contribute for the successful crossing of BBB by GBS and open new perspectives for the development of anti-infective agents in the prevention of central nervous system invasion.
Uncovering the role of IFNAR1 in Experimental Cerebral Malaria

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Abstract

Cerebral malaria (CM) is a severe complicated form of malaria resulting in an overwhelming inflammatory response in the brain due to Plasmodiumfalciparum infection. CM is an established complex and highly concurrent disease state, with many known contributors but still unknown solutions.

From human data, variants in Interferon alpha receptor-1 (IFNAR1) have been associated with protection from CM. IFNAR1 is the essential receptor for Type-I Interferon, α/β (IFN-I), which plays a complex role in regulating immune responses during infection, stimulating antigen presentation and cellular cytotoxicity, as well as eliciting protective or disease-aggravating effects. My project aims to define the role of IFNAR1 in Experimental Cerebral Malaria (ECM) using C57Bl/6 (wt) and Ifnar1-/- mice infected with Plasmodium berghei ANKA as the model. We have observed resistance to the development of ECM in Ifnar1-/- mice compared to wt mice, showing no decrease in parasite burden or disease pathology. Our results show reversal of protection from ECM upon transfer of C57BL/6 lymphocytes to Ifnar1/ mice; specifically CD8 T cells. We hypothesis that in the presence of an infection, IFNAR1 signalling contributes to ECM by eliciting CD8 T cell cytotoxic killing and/or by acting as a co-stimulatory factor in the priming of CD8 T cell response. The role of IFNAR1 is proving fundamental in the initiation of an immune response to plasmodium infection in ECM development.
Impact of common-gamma chain cytokines on human thymic regulatory T cell development

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Abstract

Natural Regulatory T cells (nTreg) constitutively expressing the transcription factor Foxp3 constitute a thymically-derived independent T cell lineage that plays a crucial role in controlling peripheral immune responses. The developmental program that thymocytes undergo to develop into mature Treg has been largely defined in mice, whereas the principles governing human Treg ontogeny are still poorly understood.

We are currently addressing the role of common-gamma chain cytokines in human Treg development by assessing their impact in both Thymic Organ Cultures and in co-cultures of total or sorted thymocyte populations and primary Thymic Epithelial Cells. Our results indicate that whereas IL-2 and IL-15 significantly enhanced the development of mature CD4 FOXP3 cells; IL-4, IL-7, IL-9 and IL-21 did not. In addition, IL-2 and IL-15 increased the expression of FOXP3 at the transcriptional level. FOXP3 cells arising in IL-2 and IL-15 supplemented cultures expressed other Treg-associated markers and displayed efficient suppressor function, as assessed in in vitrosuppression assays. Moreover, both IL-2 and IL-15 promoted the proliferation and enhanced survival of already committed Treg. Importantly, both cytokines are expressed in the human thymus as reveled by single cell flow cytometry analysis and immunohistochemistry. It is currently under investigation whether they may play a role in the commitment and/or stabilization of the Treg lineage program.

The elucidation of the molecular requirements for human Treg development should provide important insights into how to improve thymic Treg output in relevant clinical conditions, as such autoimmunity and hematopoietic stem cell transplantation.
CD6 inhibits T cell signaling

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Abstract

CD6 is a surface glycoprotein expressed on T cells, involved in the regulation of T cell development and activation, and may have a relevant role in the context of inflammatory responses and cellular expansions, given that the CD6 gene was found to be strongly associated with susceptibility to Multiple Sclerosis. Our goal is to functionally characterize CD6 during T cell activation, and determine molecular interactions of CD6 with signaling effectors. Upon engagement of T cell receptor (TCR) CD6 becomes phosphorylated on tyrosine residues suggesting that interactions with SH2 domain-containing intracellular mediators may occur. The unusual long cytoplasmic domain of CD6 additionally contains multiple potential binding sites for SH3 domain-containing signaling effectors. Our results showed that expression of CD6 in activated T cells contributes to a significant reduction in early and late T cell responses upon superantigen presentation, or TCR triggering by mAbs. Measuring calcium mobilization, we found that human primary T cells expressing CD6 reacted significantly less to APC challenge than cells where CD6 was knocked-down using siRNA or morpholinos. Calcium signals and IL-2 release were also diminished in TCR-activated Jurkat cells expressing CD6, compared to CD6 negative cells. Moreover, proliferation of blood T lymphocytes was increased when the CD6-CD166 interaction was blocked and CD6 was dispersed throughout the entire cell surface. Our data suggest that CD6 can be a signaling attenuator whose level of expression establishes the strength of signaling. However, the modulation of TCR-induced cell division and consequently the expansion of T cell populations seem to be ultimately determined by its localization at the immunological synapse.
Cytoplasmic domains of CD5 control its localization at the immunological synapse

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Abstract

T-cell receptor recognition of peptide-MHC complexes expressed on antigen presenting cells (APC) involves the formation of a tight cell-cell contact area, the immunological synapse (IS), where individual proteins are selectively partitioned. The model of synapse formation predicts an initial stabilization of the contact by engaged integrins, which anchor the region of the contact and allow the TCRs to scan MHC-peptide complexes. The T-cell surface glycoprotein CD5 targets to the IS upon T-cell binding to APC, but it has not been established whether this translocation is due to the binding of a ligand expressed in the APC or to intracellular interactions with signaling molecules or components of the cytoskeleton, or both. To dissect the molecular mechanisms responsible for CD5 localization on the IS and CD5 role in T-cell activation we have searched for cells expressing the CD5 potential ligand and identified key motifs in CD5 cytoplasmic domain. To identify cells expressing the CD5 potential ligand we have produced PE-labeled CD5 tetramers to stain a panel of different cell lines. This allowed us to clearly discriminate between CD5-ligand positive and negative cell lines. In order to determine which domains could play a role in the synapse addressing of CD5 we have used Jurkat cell lines stably expressing different CD5 mutants. Cells were incubated with superantigen-loaded APC, and following the establishment of steady interactions between the conjugating cells, we analyzed the localization of CD5 by immunofluorescence microscopy. Interestingly, when the ligand is not present, CD5 is still able to translocate to the IS, but this movement is dependent on specific sequences of its cytoplasmic tail. Our results suggest that, notwithstanding a role for the extracellular domain binding to an elusive APC-expressed ligand, a major mechanism for regulation of CD5 translocation is dependent on molecular interactions established by the CD5 cytosolic tail.
Th1-type immune response elicited in the intestinal mucosa of mice infected intragastrically with Neospora caninum

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Abstract

The protozoan Neospora caninum is responsible for clinical infections in a wide range of animal hosts. In particular, this parasite is a causative agent of abortions or stillbirths in cattle, having a major economic impact in dairy industry worldwide. Currently, no available treatment for neosporosis exists and the only commercial vaccine against bovine neosporosis showed its protective effect to be only partial.

The vertical passage of N. caninum tachyzoites from infected cows to their fetuses is a major route of infection. However, the horizontal transmission through the ingestion of sporulated oocysts also significantly contributes for neosporosis high prevalence.

The gastrointestinal (GI) tract constitutes a host first barrier to N. caninum, thus, the stimulation of the local immune response in the mucosa may constitute a privileged form of the host to counteract or avoid infection.

As the Interleukin-12/Interferon-gamma axis is essential for immune protection against this parasitic infection, we assessed here the production of these cytokines in the intestinal mucosa or mesenteric lymph nodes (MLN) of resistant C57BL/6 mice challenged with N. caninum tachyzoites (NcT) through the GI tract. Our results show that mesenteric lymph node dendritic cells (DCs) displayed an activated phenotype with up-regulated expression of surface co-stimulatory and MHC class II molecules, 18h after infection. Moreover, in the MLN, an increase in the frequency of both conventional and plasmacytoid DCs producing IL-12 was observed in the NcT challenged mice. We also observed that alpha beta IEL produced IFN-gamma early upon infection with NcT. Altogether, our results show that a Th1-type protective immune response is elicited locally in the mucosa of mice challenged with NcT. This suggests that prime boost mucosal immunization with N. caninum antigens may be worth to attempt as an alternative strategy towards vaccination against neosporosis.

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Clonal Analysis of V(D)J Rearrangement in Immunoglobulin Genes: Implications for Allelic Exclusion

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Abstract

Explaining how most B cells express only one allele from each immunoglobulin (Ig) locus remains a puzzle, after more than 40 years of empirical research. From indirect observations, such as asynchronous replication, parallels have been established between the allelic exclusion of the Ig loci and the well-known phenomenon of X-chromosome inactivation (XCI). We have asked whether the choice to rearrange first one Ig allele is determined at an early stage of development and then clonally propagated, like in XCI. To answer this question, we performed a clonal analysis of V(D)J rearrangement in vivo. Essentially, we produced mice with a hematopoietic system resulting from a single hematopoietic stem cell (HSC), which can reveal whether the individual HSC contains an imprint dictating which Ig allele recombines first. As donor mice we used F1 females with IgHa and IgHb haplotypes, that carry a single foxp3-gfp knock-in allele, an X-linked gene that works here as an internal control. Sorted IgMa and IgMb B cell populations from these mice were analyzed by PCR and V(D)J rearrangement sequencing. We found that the IgMa and IgMb populations are identical in the frequencies of different V(D)J rearrangements. We then completed this analysis by a similar type of approach in vitro, producing clones from cells at different stages of the B-cell lineage which were followed both for IgH and Igk alleles. Our Foxp3-gfp data confirm that X-chromosome inactivation is stable and clonally propagated in the hematopoietic lineage. In sharp contrast, we show that HSC do not have an early epigenetic stable imprint to rearrange one Ig allele before the other when the daughter cells become V(D)J – competent, because that scenario would have produced differences in the status of rearrangement of the silenced allele in the IgMa and IgMb B-cell pools. Allelic commitment for rearrangement, if any, starts in the multipotent progenitor to common lymphoid precursor transition. These data have implications for our current model of Ig allelic exclusion and our understanding of asynchronous replication.
Host IL-10 production is responsible for neonatal mice susceptibility against Group B Streptococcus infections

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Abstract

Group B Streptococcus (GBS) is the leading agent of severe neonatal diseases (pneumonia, septicaemia and meningitis), causing significant morbidity. Although the immaturity of the newborn immune system has been used to explain the high susceptibility against GBS infection, a suitable reason is still missing. We have identified extracellular glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a GBS factor inducing host interleukin-10 (IL-10) production early upon bacterial infection. In this study, we investigated whether the high susceptibility of neonates to GBS infection is due to their already described propensity to produce elevated amounts of IL-10. For that purpose, the serum level of IL-10 was evaluated in BALB/c pups infected intraperitoneal with 5 x 10^6 CFU of GBS NEM316. An increased in serum IL-10 concentration was observed, 1h and 4 h after infection. Moreover, the treatment with anti-IL-10R mAb, 12h before the GBS challenge, resulted in increased survival when compared with pups treated with isotype-matched control antibodies. Accordingly, we observed that IL-10 deficient (IL-10-/-) mice are also resistant against a lethal GBS infection. Severe cases of GBS-induced sepsis are characterized by a lack of neutrophil influx into infected organs. Therefore, we assessed the recruitment of neutrophil to lung and liver in infected pups. No neutrophil recruitment was observed in these infected pups. However, if the mice were treated before infection with anti-IL10R mAb, the recruitment was restored and the bacteria cleared. In addition, we found that extracellular GAPDH is responsible for the early IL-10 production observed in neonates infected with GBS and that it synergizes with TLR2 recognition of bacterial components. Antibody-mediated neutralization of GBS GAPDH or impairment of TLR2 signaling also confers neonatal protection against GBS infection by inhibiting IL-10 production and restoring neutrophil influx to infected organs. Altogether, these results indicate that the increased susceptibility of neonates against GBS infections is due to their propensity to produce higher amounts of IL-10. This is of clinical relevance since we demonstrate that once IL-10 production is neutralized, newborns are perfectly able to control infection caused by an encapsulated bacteria, despite the immaturity of their immune system.
Fetal Innate Immunity Factors reveal a protective role in a murine model of Pregnancy-Associated Malaria (PAM)

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Abstract

Malaria during pregnancy is particularly aggressive leading to decreased fetal viability, intra-uterine growth retardation, low birth weight, maternal anemia and increased infant and maternal mortality. Interaction of infected red blood cells (iRBCs) with the placental tissue evokes an inflammatory response that is characterized by myeloid and lymphoid infiltrates.

With the aim to distinguish the maternal versus fetal origin of mediators of malaria placental pathology, we generated heterogenic pregnancies targeting specific genes where KO mothers carry fetuses expressing heterozygous or KO genes and, heterozygous mothers harboring KO and heterozygous fetuses.

Making use of the C57Bl/6-NK65 experimental model recently developed in our lab, we analyzed heterogenic pregnancies for TLR4, IFNR1 and IL-10 genes. The results revealed that maternal IFNAR1 and IL-10 are detrimental for both maternal parasitemia and pregnancy outcome. Strikingly, we obtained genetic evidence that fetal TLR4 offers fetal protection which is only revealed when the mother is KO for this molecule.

We are testing the hypothesis that trophoblasts and/or fetal macrophages mediate the PAM protection effect afforded by fetal innate immunity genes.

We expect to unravel the role for the fetal components to placental pathology and PAM outcome.