SPI Workshop

Thymus @Porto

ABSTRACT BOOK

Programme

Morning session

9:15-9:30

Welcome

Claudio Sunkel - *Director of the Instituto de Biologia Molecular e Celular (IBMC)*

9:30-10:20

António Bandeira *- Institut Pasteur, Paris, France*

Phenotypic and functional heterogeneity of thymic FOXP3+ T-cells

Chairman: Iris Caramalho

10:20-11:10

Graham Anderson *- Institute for Biomedical Research, University of Birmingham, UK*

Thymus Medulla Development and Central Tolerance

Chairman: Bruno Silva-Santos

11:10-12:00

Coffee Break + poster view

12:00-12:50

Hans-Reimer Rodewald - *German Cancer Research Center (DKFZ), Heidelberg, Germany*

Thymus autonomy and T cell leukemia

Chairman: Nuno Alves

13:00-14:00

Lunch

Afternoon session

14:00-14:50

María Toribio - *Universidad Autónoma de Madrid, Madrid, Spain*

Notch1 and IL-7R signaling in human T-cell development and leukemia

Chairman: Alexandre do Carmo

14:50-15:40

Naomi Taylor - *Institut de Génétique Moléculaire de Montpellier (IGMM), Montepellier, France*

Thymus metabolism and thymus-directed therapies for T cell reconstitution

Chairman: Luís Graça

15:40-16:30

Coffee Break + poster view

16:30-17:20

Rémi Cheynier - *Institut Cochin , Paris, France*

Thymopoiesis during acute SIV infection

Chairman: Margarida Correia-Neves

17:30

Closing

Ana Espada de Sousa - *President of the Sociedade Portuguesa de Imunologia (SPI)*

Abstracts

p1

Deciphering the human FOXN1-deficiency phenotype through thymic transplantation.

Adriana Albuquerque[1], José G. Marques [2], Susana L. Silva[1,2], Dario Ligeiro[3], Blythe H. Devlin[4], Jacques Dutrieux[5], Rémi Cheynier[5], Rui M.M. Victorino[1,2], M. Louise Markert[4], Ana E Sousa[1]
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How T-cell progenitors and thymic epithelium interact to generate T-cells is incompletely understood. Forkhead box N1 (FOXN1) is a transcription factor expressed by thymic epithelium crucial for both development of the thymus and prevention of its involution. Investigation of the phenotype of FOXN1-deficiency in a patient with a homozygous R255X mutation, associated as expected with alopecia universalis, absence of thymus and T-cell immunodeficiency, unexpectedly revealed a high number of circulating T-cells displaying a regulatory T-cell-like phenotype. Importantly, the expansion of regulatory-like T-cell subset was normalized following HLA-mismatched thymic transplantation. In contrast, a large population of αβ T-cells expressing neither CD4 nor CD8 (double-negative, DN) persisted 5 years post-transplantation. Thus, our data raise the possibility that FOXN1 mutations may allow the development of a thymic rudiment that supports T-cell development, albeit with disturbances of positive/negative selection, as suggested by the expansion of DN and FoxP3+ subsets. In addition, we longitudinally quantified the sj/βTREC as a strategy to estimate intrathymic cell divisions and, consequently, thymic explant output. Our results suggest that, despite the clear achievement of a functional immune-competence, an involution of the thymus allograft occurred 3 years post-transplantation, thus, providing new insights for the design of immunological reconstitution strategies based on thymic transplantation, with potential applications in other clinical settings.

p2

Peripheral administration of foreign antigens promotes a dose dependent intrathymic selection of specific T cells

Marie-Louise Bergman, Ricardo Sousa Paiva, Santiago Zelenay and Jocelyne Demengeot.
Instituto Gulbenkian de Ciência, Oeiras, Portugal.

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 or by Thymic Epithelical Cells. We have recently established that antigen specific Treg are de novo generated in the thymus in response to pro-inflammatory peripheral immunization, indicating that peripheral antigen access the thymus and locally promote Treg differentiation.

Here, we further investigated the outcome of intrathymic selection of CD4 T cells reactive to foreign antigens injected in the periphery by assessing the relevance of the dose, and the route, of antigen administration. We report that independently of the route of antigen delivery (Foot-Pad (antigen alone, or mixed with CFA), intra-venous or even oral administration), the antigen always reaches the thymus and controls Treg differentiation. We report that Treg induction in the thymus, as well as positive and negative selection, crucially depend on the antigen dose. At low doses, although intrathymic T cells expanded significantly, no Treg differentiation was observed. At intermediated doses, we observed partial thymocyte deletion, always accompanied by Treg induction. Finally, at very high doses, thymocyte deletion was almost complete and the total number of Treg was dramatically reduced, or even totally absent. Furthermore, by analyzing various TCR Transgenic mouse models, we reveal that for each TCR, maximal Treg differentiation occurs at a specific antigen dose (probably reflecting different TCR affinities combined with the efficiency of antigen presentation).

Our findings reveal that antigens administered in the periphery can reach the thymus and determine the outcome of intrathymic T cell selection irrespective of the route of administration. This phenomenon has major implications for the understanding of how a CD4 T cell repertoire is determined, since it reveals that the antigens presented to developing T cells are not restricted to intrathymically synthesized proteins and small blood born peptides, as it has been for long assumed. Moreover, understanding this process has direct theraputical implications for the induction of both immunity and tolerance.

p3

Differentiation of human thymic regulatory T-cells at the double positive stage

Helena Nunes-Cabaço[1], Íris Caramalho[1], Nuno Sepúlveda[2,3], and Ana E. Sousa[1]
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Regulatory T (Treg) cells, best identified by the expression of the transcription factor FOXP3, play a crucial role in maintaining self-tolerance. Natural Treg cells constitute an independent thymus-derived T-cell lineage whose developmental program in humans is still ill-defined. Here we provide evidence of a Treg-cell differentiation pathway at the double positive (DP) stage, prior to commitment to the CD4+ or CD8+ lineage, in pediatric thymuses. FOXP3+ DP cells displayed a functional IL-7 receptor and increased Bcl-2 levels that may protect them from cell death/negative selection, and an activated/suppressive phenotype that was lost as CD4 single positive (SP) cells matured and acquired egress markers. A subpopulation of FOXP3+ DP thymocytes expressing CD103 likely represents the precursor of FOXP3+ CD8SP cells, which homogeneously expressed this mucosal-homing molecule. Finally, co-cultures of DP thymocytes with primary thymic epithelial cells and multiple linear regression analyses support that FOXP3+ SP cells are largely derived from FOXP3+ DP thymocytes. Overall, our data suggest that human Treg cell lineage commitment significantly occurs at the DP stage, with possible implications for the diversity and autoreactivity of the natural Treg cell repertoire.

p4

THE ROLE OF LYMPHOTOXIN-β RECEPTOR SIGNALING IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA DEVELOPMENT

Mónica T. Fernandes[1], Marinella N. Ghezzo[1], Nuno R. dos Santos[1]
[1] IBB/CBME, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

T-cell acute lymphoblastic leukemia (T-ALL) is an hematological neoplasm characterized by the malignant expansion of thymocytes, which is thought to often develop in the thymus. We hypothesize that an evolving crosstalk between leukemic thymocytes and thymic stromal cells takes place during leukemogenesis and favors its development. The lymphotoxin-β receptor (LTβR), which is expressed by thymic stromal cells and is activated by its ligands Lta1β2 and LIGHT, both expressed by thymocytes, has an important role in the crosstalk between developing thymocytes and the thymic microenvironment. In this study, we aim to understand the role of LTβR in T-ALL. As an experimental model, we use transgenic mice expressing in thymocytes the oncogene TEL-JAK2 fusion protein, which was previously found in human T-ALL. By performing quantitative RT-PCR we found higher expression levels of the genes encoding the LTα1β2 and LIGHT ligands in TEL-JAK2 leukemic T cells as compared to wild-type thymocytes. The possible mechanism underlying Lta, Ltb and Light overexpression was studied by pharmacological inhibition of selected signaling pathways, and so we found that activation of the NF-kappaB canonical pathway in TEL-JAK2 leukemic T cells, likely induces the expression of Lta, Ltb and Light genes. Supporting our hypothesis that LTβR signaling contributes to leukemogenesis, we have also found that Ltbr gene inactivation significantly delayed the onset of TEL-JAK2-induced leukemogenesis. The tumor load in the lymphoid organs and the cell surface marker phenotype of leukemic cells were, however, not significantly altered. Together, these results reveal a non-redundant role for LTβR in TEL-JAK2-induced leukemia. The mechanism by which LTβR signaling contributes to leukemogenesis is the focus of our current research.

p5

FoxN1 Expression in the Thymic Stroma Promotes T-Cell Leukemia In TEL-JAK2 Transgenic Mice;

Marinella N. Ghezzo, Mónica T. Fernandes, Nuno R. dos Santo
IBB/CBME, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

Although T-cell acute lymphoblastic leukemia (T-ALL) is a malignancy of thymocytes originating in the thymus, very little is known about the microenvironmental factors participating in this malignancy. To identify thymic microenvironmental factors that contribute to T-ALL development, we have been using a transgenic mouse model of T-ALL driven by the TEL-JAK2 fusion protein, which was found in leukemia patients. The FoxN1 transcription factor is expressed in thymic epithelial cells since the early stages of embryonic development throughout mammalian adult life, and is essential for thymic development and maintenance.. To study the role of FoxN1 in T-cell leukemogenesis we have bred TEL-JAK2 transgenic mice with mice carrying the spontaneous nude mutation of Foxn1. Two cohorts of TEL-JAK2 mice were generated, each carrying either one or two wild-type Foxn1 alleles. Our results indicate that TELJAK2 transgenic mice with only one wild-type Foxn1 allele [+/nu] develop T-cell leukemia with a statistically significant delay as compared with transgenic littermates with two wild-type Foxn1 alleles [+/+]. However, inactivation of one Foxn1 allele did not affect the tumor load, indicating that this gene is important for disease initiation. The observed difference in leukemia onset between the 2 groups of transgenic mice was not due to a reduction of cellular targets of transformation in Foxn1[+/nu] mice, because no significant difference in thymic size, cellularity, and thymocyte differentiation between [+/nu] and [+/+] mice was found. Our results indicate that FoxN1 controls the expression of genes that contribute to thymocyte transformation. Ongoing experiments aim to identify such genes.

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p6

Uncovering IL-7 thymic niche in vivo: The impact of thymocyte-thymic epithelial cell crosstalk on IL-7 expression

Ana Rosalina Ribeiro[1], Heidi Schmid[1], Pedro Rodrigues[1], Alexandra Moreira[1], Alexandre M Carmo[1], James P. Di Santo[2] and Nuno L. Alves[1]

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The capacity of our immune system to fight pathogens relies on a fully established T lymphocyte population that differentiates within the thymic environment. T cell differentiation is not a thymocyte autonomous process, being mediated by unique migratory, survival, proliferative, commitment and selection signals provided by thymic stroma. There are two known subsets of thymic epithelial cells (TECs), which differ both in function and spatial localization: cortical (cTECs) and medullary (mTEC). Both subtypes derive from a common precursor, undergoing a differentiation process that occurs simultaneously with T cell development. However, little is known about the molecular mechanisms responsible for TEC maturation.

Interleukin 7 (IL-7) is an essential thymopoietic cytokine predominantly expressed by TECs. Yet, despite our knowledge on the biological effects of IL-7 in the immune system, the nature and the developmental origin of IL-7 expressing cells, as well as the cellular and molecular mechanisms that regulate its expression, remain largely unknown. Using IL-7 reporter mice, in which yellow fluorescent protein (YFP) expression marks cells that co-express high levels of Il7 (Il7hi/YFP+TECs), we characterized this subset throughout embryonic thymic organogenesis and in adulthood under circumstances of thymic damage. We show that Il7hi/YFP+TECs emerge early during thymic development, exhibit higher proliferative status at early phases of thymic organogenesis, display phenotypic and molecular features of immature cTEC, segregates from Aire+mTECs and gradually disappear with time in a thymocyte-dependent manner. The thymocyte-mediated decrease in Il7hi/YFP+TEC occurs independently of the RANK-induced formation of mTECs. Together, our results suggest that the Il7hi/YFP+TEC subset is a thymic determinant of the cortical TEC lineage.

In addition, we show that Il7hi/YFP+ TECs, which constitute a minor TEC subset in the adult thymus, remerge upon thymic atrophy induced by g-irradiation, but not glucocorticoid treatment. The irradiation-induced re-emergence of Il7hi/YFP+ TECs is accompanied by a significant increase in Il7 expression and a restoration of cTEC population, disrupting the typical mTEC-enriched epithelial compartment of the adult thymus and recapitulating to a certain extent earlier stages of thymic development. Our findings suggest that, although the adult TEC compartment is often considered quiescent, there is a TEC subset that retains the functional plasticity to re-express Il7 upon genotoxic aggression. The extension of this stress-induced response may be taken in consideration in therapeutic approaches to boost thymic function and recovery.

p7

CD70 on dendritic and epithelial cells in the thymic medulla promotes CD4+Foxp3+ regulatory T cell development via CD27

Julie C. Ribot, Jonathan M. Coquet, Sabine Middendorp, Gerda van der Horst, Yanling Xiao, Nikolina Babala, Joana F. Neves, Daniel J. Pennington, Heinz B. Jacobs, Bruno Silva-Santos, and Jannie Borst

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.

p8

Is the control of mycobacterial growth within the thymus a job for T cells re-circulating from the periphery?

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We have recently shown in the mouse model that mycobacteria are able to infect the thymus, the organ where T cells differentiate. Upon infection with Mycobacterium avium (strain of intermediate virulence) or with Mycobacterium tuberculosis, the bacterial burden reaches a plateau by 4 weeks post-infection (wpi) in the spleen and by 3 wpi in the lung. Concurrently, the immune response peaks, characterized by an increased ability of specific T helper 1 (Th1) cells to respond to mycobacterial antigens. Interestingly, there is stabilization of the bacterial load within the thymus, indicating establishment of immunity in this organ, although it occurs at much later time-points (16 wpi). Knowing that T cells that differentiate within M. avium-infected thymi are tolerant to the invading pathogen, and that T cells do not full mature within this organ, we hypothesize that mature T cells, re-circulate from peripheral organs back to the thymus, where they participate in the control of thymic infection.

In this work, we evaluated the immune response against mycobacteria established within the thymus and assessed which T cells are involved in this process. Coinciding with the stabilization of the bacterial load in the thymus at 16 wpi, increased IFN-γ expression was observed, followed by increased iNOS expression. Moreover, at late time points, most of the mycobacteria infected cells in the thymus are iNOS+, as determined by immunofluorescence, indicating that the majority are activated. Chemokines involved in the recruitment of Th1 cells, like IP-10, MIG and MIP-1β, are up-regulated in parallel with the increased recruitment of mycobacteria-specific re-circulating T cells in the thymus. Finally, making use of T cell adoptive transfer experiments, we observed that T cells re-circulating from the periphery into infected thymi efficiently confer protection against M. avium.

This work presents evidence of an ongoing immune response in the thymus that is sustained by peripheral T cells that home back to this organ.

p9

Dissecting the mechanism(s) responsible for Mycobacterium avium infection-induced premature thymic atrophy

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Premature thymic atrophy occurs as a consequence of several pathological situations like stressful conditions, treatment with dexamethasone or estrogen, exposure to ethanol or pesticides and systemic infections. Thymic atrophy has been shown also to occur in mice during infection by several pathogens. This accelerated infection-induced thymic atrophy has been associated with different mechanisms that are not mutually exclusive like increased apoptosis of thymocytes, associated or not with increased production of glucocorticoids, and premature output of not fully differentiated T cells from the thymus.

Mycobacterium avium strain ATCC 25291 SmT causes premature thymic atrophy upon systemic infection of C57BL/6 mice. A less virulent strain, the strain 2447, also causes systemic infection but does not cause premature thymic atrophy. Taking this into account and since understanding the mechanisms responsible for thymic atrophy is essential to be able to prevent it, as well as to find a way to recover normal thymic activity, we made use of these two M. avium strains to dissect the mechanisms responsible for mycobacterial infection-induced thymic atrophy.

Our results show that the corticosterone serum levels are only slightly increased on mice infected with M. avium strain 25291. Moreover the glucocorticoid receptors are decreased and the expression of Cyp11b1 (a gene coding for an enzyme required for glucocorticoid production) is maintained in the thymus during infection by M. avium strain 25291 which suggest a minor role for corticosterone-induced apoptosis in infection-induced thymic atrophy. We also observed that infection of mice lacking the expression of interferon (IFN)-γ or the inducible nitric oxide synthetase (iNOS) with the more virulent strain does not cause premature thymic atrophy. In addiction our results show that the most immature thymocytes (DN subpopulations and early thymic precursors) are altered which suggest a role for defective bone marrow T cell precursors on premature thymic atrophy.

As future work we aim to clarify the role of each of these alterations on the mechanisms responsible for mycobacterial-infection induced thymic atrophy.

p10

Thymic Activity and Immune Reconstitution in HIV-1-Infected Patients: Looking for Reciprocity in a Longitudinal Cohort

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The introduction of Highly Active Antiretroviral Therapy (HAART) in the mid 90's was a milestone in HIV treatment. By reducing HIV-viral loads and increasing CD4+ T-cell counts in the periphery, HIV-associated morbidity and mortality has diminished enormously. However, even among virologically suppressed individuals, the pattern of CD4+ T-cell reconstitution differs. Indeed, a proportion of up to 40% HIV-infected aviremic patients present suboptimal T-cell reconstitution, the so-called discordant or immunological non-responders. Since the thymus is the organ responsible for T-cell differentiation, efficient thymopoiesis has been proposed as a relevant parameter to the replenishment of naïve peripheral CD4+ T-cell pool.

The immune reconstitution in individuals receiving HAART occurs gradually for several years. To investigate the role of thymic output in the immune system reconstitution we are performing a longitudinal study in which AIDS patients are followed from the day they initiate HAART and for the consecutive three years. Several parameters of thymic activity are currently being analysed such as CD31 expression in blood cells by flow cytometry (complemented with the routine analysis of other blood cells), determination of T-cell receptor excision circles (TRECs) and evaluation of thymic volume by computerized tomography. Additionally we are collecting clinical information on several parameters of each individual, including the lower CD4+ T-cell count, liver function and other concomitant diseases. We have currently 77 patients on our longitudinal study from which data is being collected in order to search for correlates between thymic activity and immune system restoration during HAART.

p11

CCR9 AND CCR7 ANTIGEN DEPENDENT CHANGES IN THE THYMOCYTE POPULATIONS DURING MURINE PREGNANCY

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In parallel with the well known involution that follows thymic senescence in aging, a hormonal dependent thymic involution is reported during pregnancy in rodents and humans. However, unlike aged thymus, the thymus of pregnant animals seems active despite showing several morphological changes. This phenomenon occurs in the absence of apoptotic events but together with changes in the proliferation of CD4, CD8, DP and DN thymocytes.

The chemokine receptors CCR7 and CCR9 are known to be prominently expressed by both human and mouse thymocytes and seem to be important players in the regulation of thymopoiesis.

In this study, by using CCR9 and CCR7 knockout mice we aim to study the effects of these chemokine receptors in pregnancy outcome and thymic selection during pregnancy.

Despite normal pregnancies, we observed that, contrary to the control animals, the thymocyte populations of CCR9-/- mice is not affected by pregnancy. When compared to non pregnant animals there is an increase in the population of CD4SP cells in the thymus of the CCR7-/- pregnant mice which seems to be antigen dependent. These changes were accompanied by changes in different populations of T regulatory cells and dendritic cells in the thymus and periphery.

Our preliminary results show that while pregnancy outcome is not changing in the absence of the CCR9 and CCR7 chemokine receptors, the antigenic dependent changes in different thymocyte populations during pregnancy seem to be dependent on these chemokine receptors.