HARALD VON BOEHMER
MIDWINTER
CONFERENCE
Advances in Immunobiology

CONFERENCE PROGRAM

HARALD VON BOEHMER MIDWINTER CONFERENCE 2026

Seefeld in Tirol, Austria
January 24 – 28, 2026

midwinteconference.org

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About the MWC

Dear friends and colleagues,

Welcome you to the 5th Harald von Boehmer Midwinter Conference – Advances in Immunobiology (MWC)!

The conference was initiated in 2014, when Harald von Boehmer and Ludger Klein founded the Association for the Advancement of Immunobiology, an official non-profit organization based in Munich, Germany. The aim of the Association is to provide a platform for the exchange of ideas in immunobiology and other scientific health disciplines by enabling established senior scientists and fledgling researchers to interact in a science-focused environment. The Midwinter Conference is designed to encourage open discussion, networking with peers from around the world and leveraging knowledge from leading experts in this field.

In memoriam of Harald von Boehmer, who passed in 2018, and to honor his fundamental contributions to the field of immunology, we have renamed the congress the "Harald von Boehmer Midwinter Conference".

The Harald von Boehmer MWC 2026 will feature 24 invited presentations and a similar number of talks selected from abstracts as well as two poster sessions. We are very pleased to note that the interest in the MWC continues to abound with many speakers as well as participants returning for the 3rd, 4th or even 5th time!

We sincerely hope you will enjoy your time in Seefeld,

the MWC 2026 organizing committee

Ludger Klein

Lisa von Boehmer

Thomas Brocker

Conference Speakers

Thomas Boehm
Max Planck Institute for Immunobiology and Epigenetics

Matteo Iannacone
San Raffaele Scientific Institute

Thomas Brocker
University of Munich

Nicole Joller
University of Zurich

Meinrad Busslinger
Research Institute of Molecular Pathology

Wolfgang Kastenmüller
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Willfried Ellmeier
Medical University of Vienna

Ranit Kedmi
The Weizmann Institute

Donna Farber
Columbia University

Ludger Klein
University of Munich

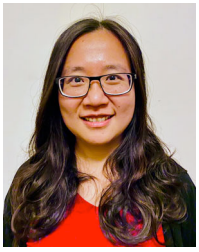
Adrian Hayday
The Francis Crick Institute

Daniela Latorre
San Raffaele Scientific Institute

Conference Speakers

Adrian Liston
The Babraham Institute

Michel Sadelain
Memorial Sloan-Kettering Cancer Center



Wan-Lin Lo
University of Utah

Georg Schett
University Erlangen-Nuremberg

David Masopust
University of Minnesota

Michael Sixt
Institute of Science and Technology Austria

Klaus Rajewsky
Max Delbrück Center

Osamu Takeuchi
Kyoto University

Caetano Reis e Sousa
The Francis Crick Institute

Naomi Taylor
National Cancer Institute

Chiara Romagnani
Charité – Universitätsmedizin Berlin

Hedda Wardemann
German Cancer Research Center

Conference Program

Saturday, January 24

KEYNOTE LECTURE AND WELCOME RECEPTION

18:00	**Klaus Rajewsky**	Growing up as an immunologist among molecular geneticists
19:00	Mix and Meet	

Conference Program

Sunday, January 25

MAKING AND SHAPING LYMPHOCYTES (1)

09:00	**Thomas Boehm**	Evolution of adaptive immunity
09:30	**Wan-Lin Lo**	Positive, negative, and everything in between
10:00	Andreas Krüger	Multi-step control of thymic selection by microRNA miR-181
10:15	Vera Martins	Limiting bone marrow chimerism impairs thymic cell competition and causes leukemia
10:30	Coffee Break	

MAKING AND SHAPING LYMPHOCYTES (2)

11:00	**Ludger Klein**	Determinants of central T cell tolerance
11:30	Andrew Koh	Vitamin D receptor modulates NF-κB bandwidth to promote thymic epithelial diversity for immunological tolerance
11:45	Gleb Turchinovic	Reconstruction of Innate Lymphoid Cell development in vitro
12:00	Lunch Break	

AT THE CROSSROADS OF INNATE AND ADAPTIVE IMMUNITY (1)

14:00	**Ranit Kedmi**	Immune tolerance to food and commensals: from induction to inflammation control
14:30	**Osamu Takeuchi**	RNA regulation in the innate immune system
15:00	**Chiara Romagnani**	Mechanisms of NK cell clonality
15:30	Coffee Break	

AT THE CROSSROADS OF INNATE AND ADAPTIVE IMMUNITY (2)

16:00	**Adrian Hayday**	Regulation of innate immunity by a prototypic adaptive immune receptor
16:30	Thomas Herrmann	Cell type specific differences of immunmodulation and human γδTCR receptor binding by Butyrophin A1
16:45	Immo Prinz	A stem-like Vδ1+ γδ T cell subset sustains effector responses to recurrent malaria infections
17:00	Thomas Winkler	A T cell receptor VDJδ knockin mouse reveals new NKT and IEL s ubpopulations of γδ T cells

Conference Program

Monday, January 26

SHAPING AND DIVERSIFYING T CELL FUNCTION

09:00	**Thomas Brocker**	Extracellular vesicles
09:30	**Wilfried Ellmeier**	Histone deacetylases and the regulation of T cell-mediated immunity
10:00	Julia Polansky	Proliferation-driven epigenetic plasticity promotes memory differentiation in human T lymphocytes
10:15	Carmen Gerlach	From T cell subsets to axes of diversification – a novel conceptual framework for understanding and measuring T cell diversification
10:30	Coffee Break	

REGULATORY T CELLS (1)

11:00	**Adrian Liston**	Molecular and cellular control of regulatory T cell tissue-residency
11:30	Thomas Korn	CD38 endows local antigen-specific Foxp3$^+$ Treg cells with stress resilience for control of compartmentalized CNS inflammation
11:45	Christina Jäger	Inducible protein degradation reveals inflammation-dependent function of the Treg cell lineage-defining transcription factor Foxp3
12:00		Lunch Break

REGULATORY T CELLS (2)

17:00	**Nicole Joller**	Regulatory T cell specialization in Th1 responses
17:30	Ari Glasner	Orthogonal targeting of CCR8+ T regulatory cells (Tregs) and the TIGIT–PVR pathway enhances anti-tumor immunity
17:45	Dominik Filipp	Safeguarding early microbiota: the emerging role of Sca-1$^+$ RORγt$^+$ regulatory T cells
18:00	Ari Waisman	Aging-associated microbiota drives Treg cell dysfunction via TNF signaling
18:15	Poster Session (1)	Enjoy fingerfood and drinks

Conference Program

Tuesday, January 27

MYELOID CELL BIOLOGY

09:00	**Michael Sixt**	Energetics of leukocyte locomotion
09:30	**Caetano Reis e Sousa**	Dead cell recognition by dendritic cells and anti-cancer immunity
10:00	Mirela Kuka	Viral context dictates a cytotoxic monocyte program that may restrain B cell responses
10:15	M. Schmidt-Supprian	Proteogenomic analyses of allergic mast cell activation
10:30	Coffee Break	

IMMUNE CELLS IN SPACE AND TIME (1)

11:00	**Donna Farber**	Establishment and maintenance of tissue resident immunity across the lifespan
11:30	**David Masopust**	Memory CD8 T cells: quantity, quality & location
12:00	Lunch Break	

IMMUNE CELLS IN SPACE AND TIME (2)

17:00	**Matteo Iannacone**	Immune surveillance of the liver
17:30	Georg Gasteiger	Strategic positioning of Tcf7hi CD8+ memory T cells in the liver enables rapid detection of systemic infection
17:45	Jan Dobes	SFB-induced intraepithelial lymhocyte differentiation: the critical molecular and cellular players
18.00	Poster Session (2)	Enjoy fingerfood and drinks

Conference Program

Wednesday, January 28

AUTOIMMUNITY

09:00	**Daniela Latorre**	Autoreactive T cells in inflammatory peripheral neuropathies
09:30	**Georg Schett**	CAR T cell therapy in autoimmune diseases
10:00	Günter Steiner	Identification of novel autoantibodies for diagnosing seronegative rheumatoid arthitis
10:15	Isabell Serr	Antigen-specific immune modulation with liver-targeting nanoparticles fosters immune protective regulatory T cells to delay Type 1 Diabetes
10:30	Coffee Break	

LYMPHOCYTE ACTIVATION AND HOMEOSTASIS

11:00	**W. Kastenmüller**	Dendritic cells initiate T cell exhaustion through a systemic feedback mechanism
11:30	Jörg Kirberg	Lymph node heterogeneity offsets competitive homeostatic exclusion among peripheral T cells
11:45	Daniel Krappmann	MALT1 alternative splicing – a molecular rheostat for tuning immune activation and homeostasis
12:00	Lunch Break	

CANCER IMMUNOLOGY

14:00	**Naomi Taylor**	Harnessing CAR precision: metabolic constraints, hinges, and fuzzy logic
14:30	**Michel Sadelain**	Evolution of natural and synthetic receptors for antigen
15:00	Alina Shomuradova	From TCR–CD3 architecture to synthetic receptors with tunable antigen discrimination
15:15	Shi Yong Neo	Nur77 agonism invigorates Natural Killer cell immunity against hepatocellular carcinoma
15:30	Coffee Break	

HUMORAL IMMUNITY AND VACCINATION

16:00	**Hedda Wardemann**	Malaria vaccine development
16:30	A. Scognamiglio	Novel encoded adjuvants for genetic vaccines
16:45	Taras Kreslavsky	Determinants of longevity, diversity and rapid responsiveness of B cell memory

FAREWELL LECTURE

17:00	**Meinrad Busslinger**	Farewell Lecture: Transcriptional Control of humoral immunity
19:00	Tyrolean Evening	

Poster Abstracts

Poster Abstracts

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Contribution of TCR Signaling Pathways to Ribosomal Biosynthesis in activated T cells

Batuhan Akçabozan, Shiva Jahani, Theresa Schnalzger, Jürgen Ruland, Thomas J. O'Neill, Daniel Krappmann, Yenkel Grinberg-Bleyer, Reinhard Obst

Ribosomal biosynthesis is a highly energy demanding process that is crucial for biomass production by highly proliferative cells like stem cells and lymphocytes. We have previously shown that it is upregulated ~9-fold by activated T cells. mTORC1, a central metabolic hub, is also a major regulator of ribosomal biosynthesis in activated T cells: rRNA synthesis was reduced by 50% in mTORC1- (i.e., Rptor-), but not mTORC2-ablated T cells. However, it remains unclear how other TCR signaling pathways contribute to the full increase of ribosomal biosynthesis. Here we show the contributions of the NF-kB and MAP kinase pathways and the roles of the mTORC1 effectors p70(S6K1/2) and p90(Rsk) to ribosomal biosynthesis and translation. Using Bcl10 and Malt1-ablated mice and inhibitors we found a ~25% reduction of rRNA synthesis. This effect is mediated mainly through mTORC1, as its activity was reduced in CBM complex ablated cells, but, so far, hardly in those lacking genes of individual NF-kB components. Experiments with T cells ablated of the mTORC1 targets p70(S6K1/2) showed that these S6 kinases are dispensable for rRNA synthesis though they showed residual phosphorylation of rpS6(235/6) and rpS6(240/4) residues, while they were completely inhibited in Rptor-ablated T cells and were susceptible to chemical inhibition of MAP kinase components. Our findings demonstrate crosstalk between CBM complex and mTORC1 signaling, delineate the distinct contributions of MAP kinase and mTORC1 pathways to S6 phosphorylation and clarify the relative contributions of CBM, NF-kB, mTORC1 and MAP kinase pathways to ribosomal biosynthesis in activated T cells.

Poster Abstracts

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Periweaning diet-induced activation of an IFNgamma-mediated regulatory circuit promotes the homeostasis of CD8+ T cells

Dogus Altunöz, Ramin Shakiba, Kaushikk Ravi Rengarajan, Hamsa Narasimhan, Sadiq Nasrah, Nikos E. Papaioannou, Jessica Vetters, Maria L. Richter, Maria Parra Reyes, Nadine Spranger, Denise Messerer, Sabine Schwamberger, Andreas Goschin, Dimitrios Starfas, Tobias Straub, Michele Proietti, Katrin Böttcher, Maria Colomé-Tatché, Dirk Haller, Jan P. Böttcher, Stephanie Ganal-Vonarburg, Sophie Janssens, Christian Schulz, Anne B. Krug, Barbara U. Schraml

Balancing pathogen defence with maintaining tolerance to benign antigens in the neonatal period is essential for survival and the establishment of life-long immune homeostasis. Instructed by environmental signals type 1 conventional dendritic cells (cDC1) drive either T cell tolerance or immunity. Here, we uncover an interferon (IFN)-gamma-driven regulatory circuit in early life that relays dietary cues to spleen cDC1. Loss-of-function demonstrates that IFNgamma-mediated STAT1-signaling induces an immunogenic maturation program in spleen cDC1 that instructs cDC1 to expand effector memory CD8+ T cells. This program emerges during weaning, when IFNgamma production from lymphocytes rises in response to chow, it occurs in germ-free mice and remains responsive to dietary intervention in adult mice. Thus, IFNgamma production from lymphocytes relays dietary information during weaning to spleen cDC1, allowing cDC1 to recalibrate the T cell pool at the moment of nutritional independence.

Poster Abstracts

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Preserving cellular identity: APOBEC2 Safeguards T-Cell Fate Integrity during lymphoid differentiation

Elias Amro, Christos Gkougkousis, Irem Telioglu, Jose Paulo Lorenzo, Joachim Seebass ,Hassan Abolhassani, Ali Bakr, Lillemor Berntson, Sandra Ruf, Charles D. Imbusch, F. Lennart Hammarström, Qiang Pan-Hammarström, F. Nina Papavasiliou

APOBEC2 is a conserved, catalytically inactive member of the AID/APOBEC family of cytidine deaminases. Unlike other family members that edit RNA or mutate DNA, APOBEC2 functions as a DNA-binding transcriptional regulator. It is highly expressed in muscle tissue, where it maintains muscle identity by repressing non-muscle gene expression. While its role in muscle is well established, our study uncovers a previously unrecognised and essential function for APOBEC2 in the immune system. We demonstrate that APOBEC2 is also expressed in hematopoietic cells and plays a crucial role in preserving lymphoid lineage fidelity. In mice, APOBEC2 deficiency leads to the emergence of aberrant lymphocytes with a hybrid identity, co-expressing both T cell and B cell genes and receptors (TCR and BCR), along with dual functional capacities. Together, our findings establish APOBEC2 as a regulator of lymphoid identity, preventing inappropriate B cell programming within the T cell lineage, with direct implications for lymphoid development and disease in both mice and humans.

Poster Abstracts

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Single-cell Mapping of Neutrophil Heterogeneity in Skin and Blood Identifies Antigen-Presenting Neutrophil Subsets in Guttate Psoriasis

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‡ Shared last authorship

Background: Neutrophils act as first responders to group A streptococcal (GAS) throat infection, which precedes guttate psoriasis (GP), an acute subtype of psoriasis, reflecting perturbed immune homeostasis. Despite recent advances in high-dimensional immune profiling, it remains unclear how neutrophil phenotypic and functional heterogeneity differs between the skin microenvironment and peripheral blood and how this shapes interactions between innate and adaptive immunity in GP pathogenesis.

Objectives: To map the phenotypic and transcriptomic landscape of distinct neutrophil subsets in GP blood and skin and identify GAS-associated neutrophil function.

Methods: We integrated high-dimensional single-cell protein and RNA expression analyses from matched blood and skin lesional samples of patients with GP and healthy controls. Comparative analyses of publicly available datasets were used for validation, while ex vivo stimulation assays, in vitro co-culture systems and cell-cell communication inference delineated immune interaction networks.

Results: We revealed significant neutrophil heterogeneity in GP, including a prominent immature subset with a partially activated phenotype enriched in the skin. This subset exhibited an antigen-presenting profile and antimicrobial features. Compared to healthy controls, GP patients showed an expansion of neutrophil subpopulations with upregulation of genes associated with migration, neutrophil extracellular trap formation, phagocytosis and antigen processing and presentation. A comparable subset emerged in healthy controls only after ex vivo GAS stimulation, and these HLA-DR+ neutrophils induced CD4+ T-cell proliferation in vitro.

Conclusions: This study provides the first detailed neutrophil characterisation in GP and highlights specific subsets that may contribute to disease pathogenesis. Antigen-presenting neutrophils may represent a novel mechanism for T cell activation in the skin following streptococcal infection and a potential target for early therapeutic intervention in progressive GP.

Poster Abstracts

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FAM83H regulates postnatal T cell development through thymic stroma organization

Ogan BM, Forstlová V, Dowling L, Šímová M, Vičíková K, Novosadová V, Špoutil F, Červenková S, Procházková M, Turečková J, Fedosieieva O, Lábaj J, Nickl P, Křížová K, Procházka J, Sedláček R and Balounová J

Family of Sequence Similarity 83H (FAM83H/ SACK1H) is primarily expressed in epithelial cells, where it interacts with casein kinase 1 (CK1) and keratins to regulate cytoskeletal organization, cell proliferation, and vesicular trafficking. Mutations in FAM83H are known to cause amelogenesis imperfecta, highlighting its critical role in enamel formation. We generated Fam83h-deficient mice (Fam83hem2(IMPC)Ccpcz, Fam83h-/-) and mice lacking a part of the N-terminal CK1-binding domain (Fam83hΔ87/Δ87). Consistent with other Fam83h-deficient models, these mice are subviable, smaller in size, and exhibit a sparse, scruffy coat, scaly skin, general weakness, and hypoactivity. Notably, both strains show impaired lymphoid cell development in early postnatal life. In the thymus, Fam83h expression is confined to thymic epithelial cells (TECs), and its deficiency in stromal cells results in disrupted thymic architecture and severe block in the expansion of DN3 (double-negative stage 3) T cells, ultimately leading to insufficient T cell production. Single-cell transcriptomic analysis reveals that Fam83h-/- cortical TECs (cTECs) express reduced levels of the TEC master regulator Foxn1, and its multiple downstream target genes, suggesting a critical role for FAM83H likely in coordination with CK1in cTEC maturation.

Poster Abstracts

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Hyperreactive B cell-induced T cell control regulates autoimmunity and lymphomagenesis

Seren Baygün, Angela Vicente Luque, Carina Diehl, Valeria Soberon, Yuanyan Chu, Marc Schmidt-Supprian

Autoimmunity and lymphomas remain major causes of morbidity. Patients with B cell–mediated autoimmune diseases have higher risk of lymphoma, and mutations in autoreactive B cells often resemble those in lymphomas, suggesting a shared etiology. TNFAIP3 (A20), a negative NF-κB regulator, is affected in both, but its role remained unclear. A20 Paradox: We found B cell–specific A20 haploinsufficiency (mild hyperreactivity) induced female-biased autoimmunity, whereas complete knockout (strong hyperreactivity) did not. Mildly hyperreactive B cells, when combined with BclxL or BAFF, caused lethal germinal center–driven autoimmunity, mimicking SLE and SjS, while complete A20 loss was protective. Combining A20 deficiency with NF-κB–inducing kinase (NIK) abolished autoimmunity, led to profound B cell depletion, and increased regulatory and effector T cells, highlighting T cell–mediated control. A20 as a Tumor Suppressor?: Despite frequent inactivation in lymphomas, neither partial nor complete A20 loss induced lymphoma, even with Bcl6-driven oncogenesis, challenging its classical tumor-suppressor role. Rheostat-Like T Cell Checkpoint: Given the altered T cell responses observed in A20-deficient models, we investigated the role of T cells and found their absence caused early mortality with autoinflammation, lymphoproliferation, and lymphoma, proportional to B cell hyperactivity. HRB cells induce cytotoxic CD8/CD4 T cells, including stem-like progenitors of exhausted T cells (Tpex). HRB cells expressing high activation markers, costimulatory molecules, and autoantigens drove highly cytotoxic T cells that efficiently killed HRB cells via Fas-FasL. Follicular lymphoma cohorts revealed patients with A20 lesions often carry mutations in immune surveillance genes, enabling lymphoma cells to evade T cell control. In this line, combining Bcl2 with A20-deficient B cells accelerated mortality in a mouse model of follicular lymphoma. These findings reveal a dose-dependent, rheostat-like control of HRB cells, linking B cell hyperactivity, T cell regulation, autoimmunity, and lymphoma

Poster Abstracts

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Deciphering the metabolic rewiring after T cell activation through CRISPR screens in primary human T cells

Julia Benz, Myriam Grotz, Rutuja Gupte, Ralf Schmidt, Ev-Marie Schuster, Kilian Schober

T cell activation is tightly coupled to metabolic rewiring to fuel elevated energy demands. In particular, T cell activation is followed by a marked increase in protein translation. However, the precise mechanisms through which metabolic pathways influence T cell activation and effector functions remain largely elusive. In this study, we used sgRNA lentiviral infection with Cas9 protein electroporation (SLICE) in primary human CD8+ T cells to perform genome-wide CRISPR/Cas9 knockout screens with two different readouts indicating cellular protein translation. Subsequent validation and in-depth functional characterization of candidate genes confirmed positive or negative regulation of protein synthesis after T cell activation. Applying CRISPR screens to investigate the metabolic dependencies of primary human T cells not only bears the potential to enhance our understanding of the molecular events governing T cell activation but also to open new avenues for the development of targeted therapies in autoimmunity, infectious diseases and cancer.

Poster Abstracts

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In situ reprogramming of dendritic cells for immunomodulation of CNS-specific Foxp3+ Treg cells

Elisa Blickberndt, Ari Waisman

The purpose of this project is to investigate the tolerance mechanisms observed in mice with EAE after applying a non-inflammatory mRNA coding for MOG. This mRNA is specifically taken up by CD11c+ cells where the MOG peptide is then expressed on MHCII (Krienke et al., 2022). When applied in mice with an active EAE, there is a decrease in MOG-specific T cells and an increase in Treg cells in the spleen. This project focuses on the mechanisms leading to tolerance induction and the cells involved. To investigate the impact of the priming phase of EAE on the tolerance induction, we apply the mRNA in an adoptive transfer EAE and, according to first results, see a mild improvement of EAE Scores in mice treated with the MOG-encoding mRNA. Additionally, we employ the uLIPSTIC mouse model which allows labeling of interaction partners based on the transfer of a biotinylated substrate from a donor to an acceptor cell. Crossing uLIPSTIC mice to a specific cre leads to the conversion of the cre-expressing cells to donor cells (Nakandakari-Higa et al., 2024). We have developed an mRNA coding for both MOG and the cre enzyme which turns only those cells that took up the mRNA into donor cells. First tests with this mRNA have shown successful transcription. In addition, we plan to analyze the cells involved in the observed tolerance induction. To do so, the mRNA will be applied in mice with an active EAE and the gene expression profile of the Tregs that infiltrate the CNS will be analyzed. A novel single cell sequencing technique called ZmanSeq will be applied (Kirschenbaum et al., 2024). ZmanSeq allows to introduce time stamps by injecting anti-CD45 antibodies coupled to different fluorophores at different time points. When sequencing the CNS cells, it can be analyzed which cells infiltrated at which time point. We have successfully established this technique in EAE since we could show the exclusion of labeling of the long-term CNS-resident microglia population even during neuroinflammation. Additionally, we could show that time stamps up to 48 hours before opening mice could still be detected by flow cytometry. These findings highlight the applicability of ZmanSeq in EAE.

Poster Abstracts

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Inflammation-driven flexibility of memory CD8+ T cell signaling

Heinrich Schlums, Wen Huang, Lars Gerullat, Ram Vinay Pandey, Mikael Sundin, Yenan T. Bryceson

T-cell activation is traditionally thought to rely on a fixed set of lineage-restricted signaling proteins. Here, we uncover an alternative signaling program in human CD8+ T-cell subsets marked by induction of PLCγ2 and stochastic acquisition of NK-like features. PLCγ2+CD8+ T-cells are antigen-experienced, proliferate rapidly in hyperinflammatory contexts, and display enhanced cytotoxic capacity but reduced cytokine production. Epigenetic profiling revealed that repression of the transcription factor BCL11B underpins this non-canonical signaling program. BCL11B deletion was sufficient to induce PLCγ2 expression, rewire signaling networks, and recapitulate in vivo phenotypes. In patients with congenital T-cell signaling deficiencies, PLCγ2+CD8+ T-cells frequently undergo further remodeling through compensatory upregulation of cognate signaling homologues. Together, we decipher inflammation-driven CD8+ T-cell adaptation, where BCL11B down-regulation diversifies T-cell receptor signaling, tunes avidity and effector functions, and potentially enables T-cell receptor-independent activation. This epigenetic flexibility may promote immune homeostasis and buffer inherited T-cell signaling defects.

Poster Abstracts

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A recirculating pool of transiently tissue-resident effector UTCs mediates early immune responses in a site-specific manner

Rupak Dey Sarkar, Johannes Siewert, Paulina Cruz de Casas, Ekaterina Kharybina, Wolfgang Kastenmüller

Unconventional T cells (UTCs)—including γδ T cells, NKT cells, and MAIT cells—bridge innate and adaptive immunity and populate non-lymphoid tissues early in development to provide rapid barrier protection. Although parabiosis studies have established UTCs as largely tissue-resident, our recent work (Ataide et al., 2022) identified a population of tissue-derived effector UTCs (eUTCs) that continuously migrate from peripheral tissues to draining lymph nodes (LNs), where they exert tissue-specific functions. However, their fate after entering LNs and the rationale behind this migration remained unclear. Using an S.aureus subcutaneous infection model, we show that eUTCs, specifically γδT cells require the draining LN as a site for TCR-dependent proliferation. By combining an eUTC reporter system, in vivo photoconversion of tissues and LNs, and scRNA-seq of eUTCs from LNs and blood, we demonstrate that eUTCs follow a defined migratory circuit: from tissues to draining LNs, then into circulation, and ultimately back to their tissue of origin. This establishes the existence of a recirculating pool of transiently tissue-resident effector UTCs. Integrating photoconversion with S.aureus infection and sterile wound-healing models, we further show that the pool of eUTCs in blood acts as a rapidly deployable reservoir that is preferentially recruited to the site of infection. These recruited eUTCs exhibit elevated effector cytokine production compared to tissue-resident UTCs, ensuring sustained neutrophil recruitment and early antimicrobial defense. These findings challenge the prevailing view that UTCs are strictly tissue-resident and uncover an unexpected migratory strategy with important functional consequences. Ongoing studies using genetic models that disrupt eUTC trafficking aim to define how this circuit shapes tissue immunity. Finally, the presence of tissue-derived UTCs in human blood highlights their potential as tissue-specific biomarkers for localized inflammation.

Poster Abstracts

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SFB-Induced Intraepithelial Lymhocytes Differentiation: The Critical Molecular and Cellular Players

Iva Pacáková, Katarína Kováčová, Tomáš Brabec, Anna Jelínková, Martin Schwarzer, Jan Dobeš

Segmented filamentous bacteria (SFB) are anaerobic, spore-forming, Clostridia-like microbes. Their adhesion triggers a strong intestinal Th17 response. Recently, we reported that naïve CD4+ T cells reactive to SFB can also differentiate into induced intraepithelial lymphocytes and express cytotoxic molecules. In parallel, the presence of SFB induces upregulation of Th1-derived IFN-γ, which in turn leads to a significant increase in MHCII expression on intestinal epithelial cells. The accumulation of SFB-specific intraepithelial lymphocytes is markedly reduced when MHCII expression on intestinal epithelial cells is ablated. Intraepithelial lymphocytes play a crucial role in maintaining gut barrier integrity, mediating tolerance to food and commensals while also mounting responses to pathogens and tumors. Despite their significance, the cellular players responsible for their induction remain poorly understood. To gain deeper insight into the differentiation pathway of intraepithelial lymphocytes, we investigated whether CD4+ SFB-specific T cells become intraepithelial lymphocytes immediately upon activation or if already activated and polarized cells later adopt the IEL fate. Our findings indicate that SFB-specific Th1 cells are particularly efficient in generating SFB-reactive intraepithelial lymphocytes. Using genetic ablation models, we further revealed that this process depends on conventional dendritic cells. Additionally, we explored other cellular players and molecular signals involved in the gut tissue adaptation of SFB-specific intraepithelial lymphocytes. This includes CSF1R+ myeloid cells, and IFN-γ signaling being essential for IELs tissue adaptation. These findings contribute to a deeper understanding of host–gut microbiota interactions and the cellular mechanisms governing the unconventional transition of CD4+ T cells into cytotoxic intraepithelial lymphocytes.

Poster Abstracts

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Immunological Signatures of Fetomaternal Tolerance in Successful and Miscarried Human Pregnancies

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Spontaneous miscarriage affects 10–20% of clinically recognized pregnancies. While genetic, anatomic, or endocrine factors explain some losses, over 50% of cases remain idiopathic. Pregnancy represents a unique immunological paradox in which the maternal immune system must establish tolerance toward the semi-allogeneic fetus. Growing evidence suggests that dysregulation of this maternal immune adaptation actively drives these unexplained losses; however, the precise mechanisms remain poorly understood. Here, we conducted comprehensive longitudinal immune profiling of peripheral blood mononuclear cells (PBMCs) collected from two cohorts, comprising 288 samples from 119 pregnant patients. The cohort was stratified into those with successful pregnancy outcomes and those who experienced spontaneous miscarriage. Samples were collected pre-conception, once per trimester, and at delivery. We used high-dimensional spectral flow cytometry to map immune dynamics and designed three comprehensive panels to assess 113 immune cell markers. This approach enabled deep quantification of subset frequencies and the expression of suppressive, activation, migration, and differentiation markers. Unsupervised clustering of longitudinal immune feature trajectories revealed distinct temporal patterns characterizing the course of gestation. In pregnancies with successful outcomes, this included a decrease in the frequency of both B cells and, unexpectedly, Treg cell populations in peripheral blood. A decline in circulating B cells may reflect their targeted recruitment or functional reprogramming at the fetal–maternal interface, where certain B-cell subsets are thought to contribute to IL-10-mediated dampening of local inflammation. Thus, misdirected B-cell trafficking could compromise the establishment of fetomaternal tolerance. Of note, we observed altered expression of multiple chemokine receptors, suggesting redirected immune cell trafficking. Comparative analysis revealed that the immune trajectories in pregnancies ending in spontaneous miscarriage diverge from this healthy baseline.

Poster Abstracts

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The role of NFκB inducing kinase in regulatory T cells

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Beyond their canonical role in suppressing immune activation, tissue-resident Foxp3+ regulatory T (Treg) cells contribute to tissue maintenance, regeneration, and repair. While the non-canonical NFκB pathway is known to regulate effector T cell maintenance, the role of non-canonical NFκB signalling mediated by NFκB-inducing kinase (NIK) in Treg development and function remains poorly defined and controversial. Here, we investigated how non-canonical NFκB signalling shapes Treg development, tissue homeostasis, and function during inflammatory disease. Using T cell transfer colitis and experimental autoimmune encephalomyelitis (EAE) models, combined with T cell- and Treg-specific NIK deletion (NIKΔT and NIKΔTreg, respectively), we assessed the role of NIK in thymically derived and peripherally maintained Tregs. Single-cell transcriptomic analyses were used to resolve tissue-specific effects of NIK on Treg heterogeneity. Deletion of NIK in all T cells significantly reduced thymic Treg precursor populations, whereas Treg-specific deletion did not impair thymic Treg development, indicating a stage-specific requirement for NIK. In peripheral tissues, NIK was essential for maintaining Helios+ effector Treg subsets and sustaining suppressive function. NIK-deficient Tregs failed to efficiently suppress colitis upon transfer into Rag-deficient hosts, resulting in severe intestinal inflammation, which was associated with impaired expansion in lymphopenic conditions. Consistently, NIKΔTreg mice exhibited increased susceptibility to EAE with elevated disease scores, indicating defective control of autoimmune inflammation. Together, these findings identify NIK as a critical regulator of effector Treg stability and function in peripheral tissues and inflammatory settings, highlighting non-canonical NFκB signalling as a key pathway in immune homeostasis and a potential target for Treg-directed therapies.

Poster Abstracts

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Safeguarding Early Microbiota: The Emerging Role of Sca-1+ RORγt+ Regulatory T Cells

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A balanced immune system relies on FoxP3+ regulatory T cells (Tregs) to prevent excessive immune responses. While thymus-derived Tregs are well known, a unique subset of microbiota-specific, peripherally induced RORγt+ Tregs has emerged as an important regulator of intestinal immunity. Our data indicate that RORγt+Tregs originate in mesenteric lymph nodes (mLNs) during early-life microbiota colonization and, remarkably, can persist long-term even in the absence of microbial antigens.This rigidity of weaning-derived RORγt+ Tregs, reflected in their long-term persistence and limited adaptability to microbial changes, is further supported by the observation that most of these cells express Sca-1, which enables precise tracking and characterization of this memory subset. Sca-1+ RORγt+ Tregs constitute up to 85% of the stable peripheral Treg population in mLNs, whereas RORγt+ Tregs generated in adulthood are largely Sca-1+ and appear less capable of contributing to the long-term pool. Interestingly, the generation of Sca-1+ RORγt+ Tregs is not strictly linked to an early ontogenetic state, i.e., the weaning period, but rather to the first exposure to the gut microbiota. This suggests a fundamental developmental distinction between early- and late-arising RORγt+ Tregs. Moreover, the presence of Sca-1 may reflect previously unrecognized properties of this subset, particularly their long-term memory stem cell characteristics. Collectively, these observations are consistent with a model in which Sca-1+ RORγt+ Tregs preserve an imprint of early beneficial microbiota, potentially contributing to long-term intestinal immune homeostasis. While further work is required to define the functional implications of this subset, this framework raises the possibility that selective modulation of RORγt+ Treg populations could, in the future, inform therapeutic strategies for dysbiosis-associated diseases.

Poster Abstracts

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Thymic B cells in central T cell tolerance

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The generation of a self-tolerant T cell repertoire during T cell development is mediated by deletion of self-reactive T cell clones or their diversion into regulatory T cells (Tregs). Dendritic cells (DCs) and medullary thymic epithelial cells (mTECs) have extensively described roles as thymic antigen presenting cells (APC) for central tolerance induction. Thymic B cells exhibit phenotypic features of activated APCs such as expression of the co-stimulatory molecule CD80, high levels of MHC II surface expression and even immunoglobulin isotope class-switching. Moreover, as previously shown by our group, a subset expresses the transcriptional co-activator autoimmune regulator (AIRE), otherwise known for its role in "promiscuous gene expression" of tissue restricted antigens by mTECs. Here, we investigated whether B cells are, next to mTECs and DCs, a similarly important APC for central tolerance induction. TCR repertoire analyses of mature thymic CD4 T cell populations in B cell deficient and B cell sufficient mice revealed a a non-redundant contribution of B cells to both clonal deletion and clonal diversion. Strikingly, the relative contribution of B cells to negative selection was quantitively similar to the corresponding impact of DCs. Intrathymic transfer of TCR retrogenic progenitor cells and experiments with transduced mature CD4+ T cells confirmed the cell-fate-specifying function of B cells. Currently, we are addressing the consequences of „escape" of B-cell–deleted TCRs and the function of B cell–dependent Treg cells using TCR transgenic mice.

Poster Abstracts

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Metabolic quiescence of naïve-like memory T cells precedes and maintains antigen-specific T-cell memory

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Metabolic activity shapes cell fate but remains challenging to capture in vivo with high resolution. We performed longitudinal metabolic and phenotypic profiling of human antigen-specific CD8+ T cells after yellow-fever vaccination on the single-cell level. During the acute phase, T cells upregulated glycolysis to fuel anabolic needs of proliferation, but predominantly used oxidative phosphorylation for energy production, as assessed via protein translation rates. Central memory T cells were the most active subset, while effector cells underwent metabolic shut-down. In contrast, weakly differentiated naïve-like memory T cells showed minimal activity, relying solely on oxidative phosphorylation already during the acute phase. Reinforcing the link between cellular quiescence and longevity, naïve-like memory cells were preferentially maintained even 26 years post vaccination. This association between differentiation degree and metabolic activity was conserved after SARS-CoV-2 vaccination and in two murine infection models. Our study dissects the metabolic profile of antigen-specific T-cell responses ex vivo, highlighting quiescence as a key feature for long-term immunological memory formation in humans.

Poster Abstracts

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From T cell subsets to axes of diversification – a novel conceptual framework for understanding and measuring T cell diversification

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Diversity within CD8+ T cell responses is commonly thought to arise from T cells differentiating into distinct subsets. While discrete subsets appear in low-parameter analyses, high-parameter data show a continuum of T cell states. Segregation of heterogeneous data into few discrete subsets may not reflect biological relevance and accounts for inconsistencies in the literature, e.g. regarding identification of effector and memory subsets. Here, we provide an alternative to T cell subsetting for measuring and explaining T cell heterogeneity. We hypothesized that distinct molecular programs drive T cell diversification in different dimensions, or 'axes', of diversification. Focusing on acute infection, we profiled virus-specific CD8+ T cells at different timepoints after infection using CITEseq. Supporting the axes of diversification framework, we identified transcriptional patterns that reflect each T cell's differentiation state – on a graded scale from stem-like to cytotoxic – irrespective of the cell's activation and proliferation state. Likewise, we identified a transcriptional signature that reflects the cell's activation state – reflecting time after TCR stimulation - irrespective of differentiation and proliferation. To extend the usability of our signatures, we developed antibody panels for flow cytometry-based computation of scores that capture a T cell's activation and differentiation state irrespective of each other. Exemplifying the added value of the axis of diversification framework beyond providing measurements of T cell state, we demonstrate that our activation score enabled the distinction of pathogen-specific CD8+ T cells from bystander memory T cells during an ongoing infection. In summary, T cell differentiation, activation and proliferation can be considered distinct axes of diversification, and measuring a cell's 'position' along each axis – for which we provide transcriptional and flow cytometry signatures - serves as cell state measurement.

Poster Abstracts

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Haploinsufficiency of PLCG2 in a Multigenerational Family Reveals Insights into Immune Dysregulation and Leukocyte signaling

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Phospholipase C gamma 2 (PLCG2) is an essential signaling protein in B cells, NK cells, and myeloid lineages. Here, we report a multigenerational family presenting with heterozygous loss-of-function mutations in PLCG2, leading to haploinsufficiency and a varying clinical phenotype in patients defined by autoinflammation, autoimmunity and recurrent infections. In-vitro and in-silico analyses suggest rapid degradation of the variant PLCG2 protein and diminished T cell receptor engagement function. Our findings support a model of PLCG2 haploinsufficiency resulting in a primary immunodeficiency with variable expressivity.

Poster Abstracts

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Orthogonal targeting of CCR8+ T regulatory cells (Tregs) and the TIGIT–PVR pathway enhances anti-tumor immunity

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Regulatory T cells (Tregs) suppress anti-tumor immunity and accumulate in solid tumors. A highly suppressive population of CCR8 positive Tregs has emerged as a selective target for tumor-resident Tregs. Here we characterized a new anti-CCR8 antibody and showed that it reduced tumor burden with efficacy comparable to systemic Treg depletion. Single-cell RNA sequencing of genetically induced KraslslG12D Trp53fl/fl lung tumors showed altered activation and exhaustion programs in CD8 T cells following C8Mab-2 treatment, including reduced exhaustion and increased memory-associated signatures. We recently showed that Treg-directed therapies can be hindered by compensatory responses in the tumor microenvironment (TME), where tissue cells activate programs that restore immune suppression or support tumor growth. Consistent with this, here we found that CCR8 targeted Treg reduction with C8Mab-2 induces a similar compensatory response, with upregulation of the TIGIT ligand PVR. TIGIT was also upregulated on CD8 T cells in the lung tumors following anti-CCR8 treatment. We hypothesized that TIGIT blockade would counter this compensatory axis and improve the outcome of CCR8 therapy. Indeed, combined C8Mab-2 and anti-TIGIT treatment showed modestly improved survival compared with either monotherapy. However, TIGIT is also highly expressed on Tregs, and not all intratumoral Tregs express CCR8. Therefore, TIGIT signaling may activate residual Tregs in the TME, a possibility consistent with recent failures of TIGIT clinical trials. We therefore hypothesized that blocking PVR would inhibit the TIGIT–PVR axis without activating residual Tregs, preserving the benefit of CCR8-targeted Treg reduction while countering its compensatory host responses. We are currently testing anti-CCR8 combined with PVR blockade. Our findings reveal a mechanism for CCR8 targeted therapy which, together with inhibition of the TIGIT–PVR axis, modulates regulatory, effector, and stromal dimensions of the tumor microenvironment. This approach offers a potential option for immunotherapy-resistant tumors and opens avenues for CCR8 orthogonal immunotherapies.

Poster Abstracts

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The Impact of Adenosine mRNA Modifications on Macrophage Function

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Macrophages are innate immune cells characterized by a high level of plasticity, which enables their quick adaptation to a changing environment required for initiating, promoting, and resolving inflammatory processes to protect from external threats and maintain tissue homeostasis. Such reprogramming cannot be achieved via transcriptional changes alone, and therefore RNA modifications on macrophages' transcripts play a crucial role in allowing such versatility needed for a fast and adaptive response. Utilizing the mouse macrophage cell line RAW 264.7 (RAW) as a model system, we investigated the impact of METTL3-mediated m6A and ADAR1-mediated adenosine-to-inosine (A-to-I) editing on macrophage pro-inflammatory activation and immune function. Mapping A-to-I editing sites using short-read Illumina sequencing and m6A using single molecule Nanopore sequencing in RAW macrophages with wild-type genotype or genetic defects in Adar1 or Mettl3, we identified RNA modification sites on more than half of the genes that are involved in macrophages' immunological functions. Transcriptomic analysis identified ADAR1's and METTL3's crucial role in macrophage activation in response to pro-inflammatory stimuli. Using functional assays, we confirmed METTL3's and ADAR1's impact on macrophage activation such as the upregulation and presentation of immunostimulatory cell surface markers and phagocytic activity. While m6A levels remained mostly stable upon ADAR1 depletion, loss of METTL3 globally decreased A-to-I editing levels after pro-inflammatory stimulation. We observed a regulatory effect of m6A sites on the Adar1 transcript that impact Adar1's splicing and translation into protein. Additionally, we found a distance-dependent negative association between A-to-I and m6A when the different modifications occurred in close proximity which was confirmed by single-read analysis. Together, we demonstrated the importance of METTL3 and ADAR1 for macrophage pro-inflammatory function while observing different layers of cross-talk between the two mRNA modifications.

Poster Abstracts

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Human intestinal epithelium orchestrates tissue-resident T cell response to Salmonella

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The intestine harbors the largest reservoir of immune cells in the body, with tissue-resident memory T cells (TRMs) as frontline sentinels. While mouse models have demonstrated the importance of TRMs in responding to intestinal pathogens, human TRM responses to bacterial infections remain poorly understood due to limited access to patient-derived material and the absence of physiological human model systems. Notably, the role of the intestinal epithelium in governing TRM activation, particularly through non-cognate mechanisms, has been underexplored. To address these challenges, we developed fully human, organoid-derived intestinal tissues comprising a topographically accurate, multilineage epithelium with a mucus layer and autologous, intraepithelial TRMs. This tri-compartmental system enabled luminal infection with Salmonella Typhimurium (S.Tm), allowing us to identify an epithelial gene regulatory network mediating the response to S.Tm. Our findings show that epithelial-secreted IL-18 serves as primary cytokine acting on CD4+ TRMs upon infection, with these cells driving pro-inflammatory responses and epithelial damage. CD4+ TRMs exhibited high IL-18Rα expression, in contrast to CD8+ TRMs and blood-derived T cells; the latter not responsive upon infection. Using virulent and avirulent S.Tm strains, we confirmed that epithelial IL-18 secretion and subsequent TRM activation correlate with S.Tm invasion and virulence. Further, CRISPR-mediated epithelial IL-18 knockouts demonstrated that the CD4+ TRM-driven immune response is dependent on IL-18, reducing pro-inflammatory cytokine production beyond type I cytokines, and limiting excessive inflammation. In summary, we show that intestinal epithelial cells can orchestrate anti-bacterial immunity by non-cognate activation of the TRM compartment showcasing the potent innate-like functionalities of human TRMs. Our study provides one of the most complex in vitro intestinal models to date, enabling mechanistic dissection of human epithelial-immune interactions and host-pathogen dynamics in intestinal homeostasis and infection.

Poster Abstracts

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Cell type specific differences of immunmodulation and human γδTCR receptor binding by Butyrophin 2A1

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1-5% of human T cells express Vγ9Vδ2 TCR. These TCR recognize Butyrophilin (BTN) complexes formed after intracellular binding of phosphorylated intermediates of isoprenoid synthesis (Phosphoantigens; PAg) to BTN3A1. This binding leads then to formation of complexes of BTN2A1 homodimers and BTN3A1-A2 heterodimers which serve as Vγ9Vδ2 TCR activating ligand engaging CDRs, HV4 and framework regions of both TCR chains while BTN2A1 alone binds to Vγ9 germ line encoded sequences. PAg accumulate in infected and amino bisphosphonate (e.g. Zoledronate) treated host cells and in some tumors allowing the Vγ9Vδ2 TCR to act as sensor of pathological metabolic changes of PAg levels in host cell. This and the NK/CTL like effector functions provide a rational for analysis of the immunotherapeutic potential of Vγ9Vδ2T cells which is highlighted by promising data of a recent phase I/II trial in acute myeloic leukemia (AML). Mechanistic studies allowed us to identify BTN2A1 as mandatory for the PAg-response but also to show that its over-expression in 293T cells significantly reduces the Vγ9Vδ2 TCR mediated PAg response. This suppression was even more pronounced for 293T cells expressing chimeric BTN3A- molecules with an N-terminal BTN2A1-V domain, indicating its pivotal role in suppression. Importantly, overexpression of BTN2A1 in the RAJI B cell lymphoma cells showed no such effect, suggesting a dual role of BTN2A1 as an enabler for a Vγ9Vδ2 T cell response to PAg but also as potential suppressor or terminator of such a response, which is probably caused by cell type specific post-translational modifications. This Janus-faced function needs to be considered when interpreting BTN(2A1) expression data, especially on tumors and other pathological conditions. Post-translational modifications are also the likely cause of differential Vγ9Vδ2 TCR-binding ofBTN2A1-Fc constructs with identical aa sequence but generated under different cell culture conditions. A variation which is of special interest given that BTN-Fc constructs are commonly used to search for BTN-binding partners and to analyze the physiology of BTNs as immunomodulators.

Poster Abstracts

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Refractory DC promotes CD8 T cell exhaustion

Anfei Huang, Milas Ugur, Martin Vaeth, Yinming Liang, Georg Gasteiger and Wolfgang Kastenmüller

Upon infection, emergency myelopoiesis (EM) profoundly remodels the production of neutrophils and monocytes thereby amplifying innate immune defense mechanisms. Here, we investigated how EM impacts on the development and function of dendritic cells (DCs) and, thus on adaptive antiviral immunity. Five days after infection, DC arising from EM predominated in lymphoid organs, yet were refractory and responded poorly to pathogen-associated molecular patterns. Functionally, refractory DC that presented viral antigens induced CD8 T cell exhaustion and consequently provided essential protection against fatal immunopathology. Mechanistically, we identified DUSP-1 as a key regulator that mediates the refractory state of DC in mice, and found it upregulated in DCs from convalescent COVID-19 patients, suggesting a conserved role in humans. Together our study proposes that CD8 T cell exhaustion is actively orchestrated by the immune system through a systemic negative feedback loop, thus providing new angles for immunotherapeutic intervention in chronic viral infection and cancer.

Poster Abstracts

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Emergency dendritic cell poiesis drives CD8 T cell exhaustion

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Upon infection, emergency myelopoiesis (EM) profoundly remodels the production of neutrophils and monocytes thereby amplifying innate immune defense mechanisms. Here, we investigated how EM impacts on the development and function of dendritic cells (DCs) and, thus on adaptive antiviral immunity. Five days after infection, DC arising from EM predominated in lymphoid organs, yet were refractory and responded poorly to pathogen-associated molecular patterns. Functionally, refractory DC that presented viral antigens induced CD8 T cell exhaustion and consequently provided essential protection against fatal immunopathology. Mechanistically, we identified DUSP-1 as a key regulator that mediates the refractory state of DC in mice, and found it upregulated in DCs from convalescent COVID-19 patients, suggesting a conserved role in humans. Together our study proposes that CD8 T cell exhaustion is actively orchestrated by the immune system through a systemic negative feedback loop, thus providing new angles for immunotherapeutic intervention in chronic viral infection and cancer.

Poster Abstracts

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Inducible protein degradation reveals inflammation-dependent function of the Treg cell lineage–defining transcription factor Foxp3

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Regulatory T cells (Treg cells) are immunosuppressive CD4 T cells defined by expression of the transcription factor Foxp3. Genetic loss-of-function mutations in Foxp3 cause lethal multiorgan autoimmune inflammation resulting from defects in Treg cell development and suppressive activity. Whether Treg cells are continuously dependent on Foxp3 is still unclear. Here, we leveraged chemically induced protein degradation to show that functionally suppressive Treg cells in healthy organs can persist in the near-complete absence of Foxp3 protein for at least 10 days. Conversely, Treg cells responding to type 1 inflammation in settings of autoimmunity, viral infection, or cancer were selectively lost upon Foxp3 protein depletion. Acute degradation experiments revealed that Foxp3 acts mostly as a direct transcriptional repressor and modulates responsiveness to cytokine stimulation. This inflammation-dependent requirement for continuous Foxp3 activity enabled induction of a selective antitumor immune response upon systemic Foxp3 depletion, without causing deleterious T cell expansion in healthy organs.

Poster Abstracts

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Deconvolution of the Immunomodulatory Landscape in High-Grade Serous Ovarian Cancer at Single-Cell Resolution

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Adoptive T-cell therapy remains challenged by the immunosuppressive tumor microenvironment (TME) of solid tumors such as ovarian cancer. To gain a systems-level understanding of this barrier, we manually curated a database of T and NK cell modulatory proteins and analyzed their expression across single-cell RNA sequencing datasets from 76 high-grade serous ovarian cancer (HGSOC) patients. Our study uncovers a suite of known and novel immune regulators, including inhibitory ligands, adhesion molecules, chemokines, and secreted proteins, which collectively shape the dysfunctional immune landscape. Crucially, we deconvoluted the specific cellular compartments responsible for producing these immunomodulatory factors. These findings provide a broad and actionable understanding of the immunosuppressive mechanisms in the TME, offering a resource to guide future therapeutic strategies.

Poster Abstracts

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Development and function of effector Tregs

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Regulatory T cells (Tregs) are central to immune homeostasis, immune regulation, and tissue function. Following thymic development, Tregs distribute to lymphoid and non-lymphoid tissues. Within lymphoid organs, two types of Treg can be detected: central Tregs (cTregs) that recirculate between lymphoid tissues, and effector Tregs (eTregs) that circulate between peripheral tissues. Although it has been proposed that eTreg precursors transit through lymphoid tissues prior to tissue entry, functional evidence for this pathway – and for distinct developmental trajectories of eTreg subsets – remains limited. Using fate mapping mouse models, we found that eTreg continuously develop from cTreg, as well as uncover developmental relations between eTreg subsets, which can be further discriminated based on IL18R and IL33R expression. By selective depletion of IL18R+ eTreg, we demonstrate their critical role for immune homeostasis, and in regulating adaptive immune responses in the context of viral infections, asthma, and tumour. In summary, we unveiled the developmental trajectory of eTreg subsets, and identify function of IL18R+ eTreg in various immune challenges.

Poster Abstracts

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Lymph node heterogeneity offsets competitive homeostatic exclusion among peripheral T cells

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Placing mature monoclonal T cells into an environment pre-occupied by monoclonal T cells of a different specificity yields results that depend on the chosen TCRs: Either donor T cells show homeostatic proliferation within the adoptive recipient and the experiment can be reciprocated (same outcome when reversing donor / recipient). Or in one of the reciprocal experiments no homeostatic proliferation occurs, demonstrating that for these TCRs there is an apparent hierarchy. Interestingly, in the latter outcome the competitive exclusion from homeostatic proliferation may occur across MHC-restriction. This shows that although the hierarchy is defined by the given TCRs, soluble ligands must be involved (e.g. by competition / signaling-strength modulation for IL 7). To explain these opposing outcomes, one may postulate that ‚escape' from competitive exclusion is a localized phenomenon. In this, different lymph nodes vary in MHC-presentation of (tissue-specific) self-antigens that, for some TCRs, may represent the relevant ligand in triggering homeostatic proliferation. Indeed, variances among different lymph nodes were revealed experimentally (Fugmann et al. JI 2017; Nanaware et al. Cell Rep 2024). Supporting this notion, homeostatic proliferation, CD69 expression, and/or temporal retention occurred in a localized way for T cells of HA-TCR and OTII TCR transgenic lines. Finally, using a strain in which all TCR-diversity relies on one TCRα allele, in independent individuals particular TCRs became enriched in specific lymph nodes when blocking lymphocyte recirculation. These results directly demonstrate that T cells perceive distinct TCR-signaling strength in different lymph nodes. Thus, different lymph nodes constitute dissimilar niches and such variances are expected to contribute to the peripheral upkeeping of a diverse TCR-repertoire.

Poster Abstracts

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Vitamin D receptor modulates NF-κB bandwidth to promote thymic epithelial diversity for immunological tolerance

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Self-reactivity of the T cell repertoire established in the thymus varies across individuals to contribute to autoimmunity risk and host defense. Ectopic expression of tissue-specific genes in medullary thymic epithelial cells (mTECs) plays a central role; however, the determinants underlying the variability of this process remain unclear. Here, we investigated the influence of nuclear receptors that sense cell-extrinsic factors – such as nutrients and hormones – on the diversity of self-antigens expressed by mTECs for central tolerance induction. Using a single-cell multiomics approach, we identified unusually high vitamin D receptor (VDR) expression and activity levels in mTECs. Conditional deletion of Vdr limited transcriptional diversity across mTECs, causing select cohorts of tissue-specific genes to be prioritized at the expense of hundreds of others becoming silenced. This compromised mTEC diversity led to the escape of self-reactive T cells from thymic selection and multi-organ autoimmunity. By single-cell chromatin profiling, we found VDR-deficiency enhanced NF-κB activity in mTECs, particularly at putative enhancers near the 'prioritized' genes which were selectively enriched with NF-κB target motifs. Moreover, VDR directly bound to and repressed chromatin accessibility at these putative enhancers to inhibit NF-κB localization, allowing redistribution of NF-κB to alternative sites that increased the range of ectopic gene expression for central tolerance induction. Taken together, our findings define VDR as a modulator of mTEC transcriptional diversity and provide a potential mechanistic link between vitamin D deficiency and increased susceptibility to autoimmune diseases.

Poster Abstracts

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CD38 endows local antigen-specific Foxp3+ Treg cells with stress resilience for control of compartmentalized CNS inflammation

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Foxp3-expressing regulatory T (Treg) cells protect from systemic autoimmunity. However, little is known about the significance of Treg cells in inflammation-experienced tissues. Using experimental autoimmune encephalomyelitis (EAE), we show that Treg cells accumulate and persist in the central nervous system (CNS) long after the resolution of the bulk of the inflammatory infiltrate. CNS-specific local depletion of post-inflammatory Treg cells, but not systemic depletion of Treg cells, immediately rekindles autoimmune inflammatory flares in the CNS by residual local effector T cells. Expression of the NAD-consuming ecto-enzyme CD38 is crucial for the functional adaptation of post-inflammatory CNS Treg cells to a stressful microenvironment, in which access to IL-2 is limited. CD38 counteracts ADP-ribosylation of the IL-2R and, thus, maintains its high sensitivity to IL-2. Their fully functional high-affinity IL-2 receptor prevents the loss of tissue-resident antigen-specific Treg cells. These „stress-tolerant" CNS Treg cells impede the collapse of immune homeostasis in the CNS once acute inflammation is controlled.

Poster Abstracts

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MALT1 alternative splicing – a molecular rheostat for tuning immune activation and homeostasis

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MALT1-TRAF6 interaction drives lymphocyte activation and adaptive immunity, but it also contributes to maintaining immune homeostasis. While the MALT1A isoform contains two TRAF6 binding motifs (T6BM1/2), alternatively spliced MALT1B only contains the second T6BM2. We recently identified the homozygous human germline mutation MALT1 E806D, which exclusively impairs TRAF6 binding to MALT1B, and is associated with an immune disorder that combines symptoms of immune deficiency and autoimmunity in the adolescent patient. To study the etiology of this complex disease and the contribution of TRAF6 binding to alternative MALT1 splice variants, we introduced the orthologous MALT1 E814D mutation into mice. Surprisingly, homozygous Malt1 E814D mice develop within 3 weeks after birth a fatal autoimmune inflammation, which is accompanied by erythrocytes loss and liver destruction. We demonstrate that differences in alternative splicing of MALT1 provoke a severely decreased MALT1A expression in murine compared to human immune cells. By genetic engineering, re-enforced MALT1A expression yielded MALT1 A-ED mice, which are protected from the fatal immune pathology at young age. However, upon aging all heterozygous mice with only one functional T6BM and thus suboptimal MALT1-TRAF6 interaction, develop autoimmune symptoms that resemble the human immune pathology associated with the MALT1 E806D mutation. Thus, cross-species comparisons provide evidence that MALT1 alternative splicing tunes the level of TRAF6 binding, thereby functioning as a molecular rheostat in immune signaling that balances between optimal immune activation and maintenance of immune homeostasis.

Poster Abstracts

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Investigating mechanism of protection from experimental autoimmune encephalo myelitis during pregnancy

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Pregnancy has a profound impact on the course of several autoimmune pathologies. A large body of clinical research has identified a notable reduction in disease activity of Multiple Sclerosis (MS) and Rheumatoid Arthritis (RA) during pregnancy, particularly during the third trimester. This is followed by a spike in disease activity post-partum. This phenomenon has led researchers to explore hormonal therapies aimed at emulating the immune status during pregnancy. However, a phase 2 clinical trial employing estriol failed to show a clear benefit for MS patients. A study in mice has shown that pregnant animals are resistant to the induction of experimental autoimmune encephalomyelitis (EAE) during but not after pregnancy. The study proposed that during the priming phase of disease, a relative increase in frequency of regulatory T cells (Tregs) blunts activation of autoreactive cells, as Tregs are more resistant to Progesterone-induced cell death compared to conventional CD4 T cells. As Treg therapy has shown limited efficacy in EAE, we wondered whether other mechanisms may also be in play. Herein, we found pregnant mice to be completely protected from active EAE, but only partially from adoptive transfer EAE. Moreover, we found no difference in disease severity between recipient mice receiving encephalitogenic T cells raised in non-pregnant or pregnant donors. Through unbiased analysis of immune populations before EAE onset in the inguinal and cervical lymph nodes as well as the CNS, we found the most significant changes between pregnant and non-pregnant mice in the CNS, with pregnant mice showing lower number of infiltrating T cells, reduced cytokine and tissue-residency marker expression within. We therefore hypothesize that the main mechanism of EAE protection in pregnancy occurs during the effector phase of the disease and not during the expansion and activation of autoimmune T cells. We are in the process to further delineate the underpinnings of how pregnancy influences immunopathology in the CNS.

Poster Abstracts

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Determinants of Longevity, Diversity and Rapid Responsiveness of B Cell Memory

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A hallmark of adaptive immunity is its ability to generate long-lasting memory that protects against reinfections. In humoral responses, memory is maintained by plasma cells and memory B-cells (MBCs), with a diverse pool of MBCs being important for responses to new pathogen variants. Mechanisms underlying MBCs' longevity, rapid responsiveness, and diversity remain largely unknown. MBCs can arise either directly from early activated B-cells or via the germinal center (GC) reaction. Non-GC-MBCs were viewed as a minor, insignificant subset. Our recent work challenged this view, showing that non-GC-MBCs outnumber GC-MBCs across all tested scenarios. Our unpublished results suggest that these cells exhibit greater diversity and, unlike GC-MBCs, can efficiently enter secondary GCs to refine specificity, making them uniquely equipped to respond to new antigenic variants. To start testing this possibility, we have now developed a novel Cre/Dre dual fate-mapping system that enables side-by-side comparison of the MBCs generated by the two pathways. While characterizing the MBC compartment, we identified a large population of splenic MBCs with the signature of marginal zone (MZ) B cells. MZB cells are viewed as a long-lived, innate-like lineage of naïve B-cells (distinct from conventional follicular (FO) B-cells) strategically positioned in the spleen's MZ for rapid responses to bloodborne threats. Unlike naïve MZB-cells, the origin and functions of MZ-MBCs were unknown. We show that these cells originate from both MZ and FO naïve B cells across diverse immunization scenarios, challenging the notion of MZB cells as a stable lineage. In contrast to FO-MBCs, which decline over time due to apoptosis, MZ-MBCs' numbers remain remarkably stable, suggesting that MZ acts as a long-term survival niche. Access to this longevity niche is open to MBCs generated in lymphoid organs other than the spleen. Genetic ablation of MZ-MBCs impairs rapid recall responses to bloodborne challenges. Thus, splenic MZ serves as a long-term niche for MBC survival critical for rapid recall responses to bloodborne threats.

Poster Abstracts

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Multi-step control of thymic selection by microRNA miR-181

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MicroRNAs (miRNAs) are critical post-transcriptional regulators that have been implicated in governing multiple steps in T-cell development. They act by guiding RNA-induced silencing complexes to miRNA response elements (MREs) in target mRNAs, inducing translational inhibition and/or mRNA degradation. A substantial excess in putative MREs when compared to the concentration of their corresponding miRNAs constitutes a major challenge in understanding the molecular mechanisms underlying miRNA-mediated regulation. Here, we generate a high-resolution map of miR-181a/b-1 (miR-181) MREs to define the targeting rules of miR-181 in developing murine T cells. By combining a multi-omics approach with computational high-resolution analyses, we uncover novel miR-181 targets. Functional studies reveal a multi-level role of miR-181 in thymic selection of conventional T cells by modulating both death by neglect in positive selection and TCR signaling thresholds to control negative selection. In conclusion, deep profiling of MREs in combination with functional validation revealed a novel layer of posttranscriptional control of thymic selection.

Poster Abstracts

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RIG-I-like receptor-dependent type I Interferon regulates antigen dose and activation in yellow fever vaccine 17D-infected antigen presenting cells

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The live-attenuated yellow fever vaccine 17D-204 (YF17D) activates robust innate immune responses followed by rapid induction of adaptive immunity resulting in long-lasting protection. YF17D triggers the production of type I interferons (IFNs) which have a dual role in antigen presenting cells regulating their infection and contributing to their activation. Infection with YF17D was detected in primary human blood monocytes and conventional dendritic cells (DCs) and in monocyte-derived DCs but was highly restricted by type I IFN. Blocking IFNAR signaling in YF17D-infected PBMC from vaccinated donors resulted in increased activation of YF17D-specific CD8+ T cells. Consistently, peak IFN-alpha plasma levels correlated inversely with the CD8+ T cells response in YF17D vaccinees. Loss of function experiments demonstrated a dominant role of retinoic acid inducible gene I (RIG-I)-like receptors (RLRs) and mitochondrial antiviral signaling protein (MAVS) for type I IFN induction and restriction of YF17D. The type I IFN response was mediated by 5' tri- or diphosphate dsRNA intermediates that are formed during YF17D infection. In vivo proximity labelling (IPL) of RIG-I and next-generation sequencing confirmed interaction of RIG-I with YF17D-dsRNA in infected cells. Thus, YF17D-triggered RLR-signaling restricts viral replication through type I IFN and thus limits the production of viral antigens that can be presented to T cells.

Poster Abstracts

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Viral Context Dictates a Cytotoxic Monocyte Program that May Restrain B Cell Responses

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Monocytes exert context-dependent effects during infection, either promoting or restraining immune responses. We previously demonstrated that during non-cytopathic lymphocytic choriomeningitis virus (LCMV) infection, monocytes suppress B-cell responses and limit antiviral antibody production; however, the underlying molecular mechanisms remain poorly defined. To elucidate these suppressive pathways, we comprehensively characterized the phenotype of LCMV-recruited monocytes using transcriptomic analyses, flow cytometry, and confocal microscopy. Bulk and single-cell RNA sequencing revealed that a distinct subset of monocytes recruited to the draining lymph nodes (dLNs) during infection upregulates genes encoding the cytotoxic effector molecules granzyme A (GzmA) and granzyme B (GzmB) over the course of infection. Flow cytometric and confocal imaging analyses confirmed that a fraction of these monocytes express GzmA, GzmB, and perforin (Prf1) at the protein level and localize in close proximity to LCMV-specific B cells within the dLNs. Induction of this cytotoxic program in monocytes was dependent on type I interferon signaling, consistent with previous reports—including our own—linking type I interferons to impaired B-cell responses during viral infection. Importantly, preliminary functional studies indicate that this cytotoxic signature is biologically relevant: pharmacological inhibition of GzmA or conditional deficiency of Prf1 resulted in enhanced survival of LCMV-specific B cells. These findings suggest that monocyte-derived granzymes and perforin might directly contribute to the suppression of antigen-specific B-cell responses. Ongoing studies aim to validate this mechanism and further define the molecular and functional identity of this suppressive monocyte subset. Elucidating this unexpected monocyte function may reveal novel viral strategies for subverting humoral immunity and promoting persistent infection.

Poster Abstracts

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Regulatory T cells impairments evolve prior to the onset of beta cell autoimmunity

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In autoimmune Type 1 Diabetes (T1D), impaired self-tolerance promotes immune-mediated destruction of insulin-producing β-cells resulting in insulin deficiency. The disease progresses through distinct stages, with genetic factors determining disease susceptibility. The clinical manifestation is preceded by an asymptomatic phase termed islet autoimmunity. Regulatory T cells (Tregs) mediate immunological self-tolerance and have been shown to be essential in preventing autoimmunity and limiting chronic inflammatory diseases. Impairments in Treg function, induction and stability were found at later stages of islet autoimmunity and in clinical T1D. Especially Treg instability is hypothesized to be a consequence of autoimmune activation and the proinflammatory environment during autoimmunity. In contrast to this hypothesis, we observed Treg impairments before islet autoimmunity onset in infants with high genetic risk and in NOD mice, an established mouse model for T1D. We showed impaired in vitro Treg induction in young NOD mice (age < 30 days) before and after islet autoimmunity onset compared to non-autoimmune prone BALB/c mice ($p<0.001$). Furthermore, we identified an unstable Treg population that was present before the onset of islet autoimmunity in NOD mice and in infants with high genetic T1D risk (>10 % genetic risk). This Treg population exhibited typical instability markers, including loss of Foxp3 and increased Foxp3 CNS2 methylation. Furthermore, Tregs from at-risk infants and NOD mice with early islet autoimmunity secreted higher amounts of proinflammatory IFN-γ ($p<0.05$), indicative of aberrant Treg plasticity. These findings provide evidence that Treg impairments occur very early in T1D pathogenesis, indicating a possible causative role in disease initiation, autoimmune activation and progression. The results underscore the importance of early immunological impairments for T1D pathogenesis and highlight the need for preventive intervention strategies in children at risk for developing T1D.

Poster Abstracts

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Cellular mechanisms underlying antibody-mediated immune responses in the upper respiratory tract

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Nasal vaccination induces potent mucosal immunity, yet the cellular origins, trafficking cues, and microanatomical niches that support IgA production in the upper airways remain incompletely defined. Here, we integrate structural, cellular, and molecular analyses to delineate how antigen-specific B cell responses are initiated and sustained in nasal-associated lymphoid tissue (NALT) and subsequently embedded within the nasal turbinates. Using intact-organ imaging, we show that nasal vaccination triggers B cell expansion in the NALT subepithelial dome, where pre-activated antigen-specific T cells interact with cognate B cells in interfollicular regions to initiate germinal center (GC) activity. Effective GC seeding requires both sufficient B cell receptor (BCR) affinity and CCR6 upregulation; low-affinity B cells fail to induce CCR6, do not support T follicular helper cell differentiation, and cannot enter NALT GCs. CCR6 deficiency likewise prevents B cell translocation to the subepithelial dome and blocks class-switch recombination to IgA in response to immunization or commensal signals. Following GC differentiation, NALT-derived IgA+ B cells rely on PSGL-1–mediated interactions with endothelial selectins to home through the circulation to the nasal turbinates. There, glandular acinus structures provide a specialized immunological niche that recruits and positions IgA-secreting plasma cells. Vaccination enhances CCL28 expression in the turbinates, promoting selective homing of IgA+ cells to these sites. NALT ablation disrupts this entire pathway, confirming its central role as the upstream generator of IgA-secreting effector cells. Together, these findings reveal a coordinated program in which BCR affinity, CCR6-dependent localization, and chemokine-driven trafficking shape IgA immunity across NALT and turbinate niches. This integrated understanding highlights actionable targets—such as modulation of CCR6 expression, enhancement of T–B cell priming, and tuning of chemokine cues—for optimizing nasal vaccine design and potentially treating disorders of upper airway immunity.

Poster Abstracts

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Limiting bone marrow chimerism impairs thymic cell competition and causes leukemia

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Cell competition in the thymus is a critical tumor suppressor mechanism that prevents leukemia. Impairing this process enable thymocyte self-renewal, resulting in a phenomenon termed thymus autonomy, which ultimately contributes to the development of T cell acute lymphoblastic leukemia (T-ALL). Here, we show that suboptimal bone marrow reconstitution in γc-deficient mice initiates thymus autonomy and subsequent T-ALL. In such conditions, DN3-early thymocytes increased their proliferation rates and CD4+CD8+ double positive-like cells lacking a TCRβ chain emerged. These populations also emerged from Rag-deficient progenitors, despite their differentiation block at the DN3-early stage. Of note, culture of progenitors prior to their injection affected incidence and onset of T-ALL. Moreover, inneficient bone marrow reconstitution results in intermittent T lymphocyte production, consistent with sporadic thymic colonization events. The structural integrity of the thymus and its capacity to support thymopoiesis, significantly impacts leukemogenesis. We demonstrate that there is a threshold for effective bone marrow reconstitution that is critical for reinstating thymic cell competition and inhibiting thymus autonomy. Collectively, our data reveal that thymus autonomy precedes T-ALL development following inefficient bone marrow correction of γc-deficiency, underscoring the need for therapeutic strategies that mitigate thymus autonomy while effectively correcting immunodeficiency.

Poster Abstracts

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FCRL3 is an immunoregulatory receptor that restrains the activation of human memory T lymphocytes

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Genetic variants in the FCRL3 gene are linked to autoimmune disorders. However, the functional properties of FCRL3- expressing T lymphocytes, and the regulation and functional impact of FCRL3 expression remain understudied. Here, we performed a multiomic and functional analysis of human T lymphocytes expressing FCRL3. FCRL3 expression correlated with reduced capacity of T cells to undergo activation and was accompanied by functional specialization toward a cytotoxic phenotype, resembling cytotoxic CD4+ T lymphocytes and CD8+ effector memory TEMRA cells. FCRL3 expression was induced upon repetitive TCR engagement, and sufficed to attenuate T cell responses, indicating a role as a negative regulator of the activation of differentiated T cell subsets with high cytotoxic capacity. Mechanistically, the cytoplasmic domain of FCRL3 engaged inhibitory molecules, suggesting a direct role in limiting activating signals. Overall, our study establishes FCRL3 as a functional immunoregulatory receptor that restrains the activation of highly specialized human memory T cells.

Poster Abstracts

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Nur77 agonism invigorates Natural Killer cell immunity against hepatocellular carcinoma

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Despite promising development as emerging "off-the-shelf" therapeutics against cancer, natural killer (NK) cells still faced considerable challenges in the solid tumor microenvironment (TME) including poor penetrance and immuno-suppression. Here, we employed spatial and single-cell transcriptomics to reveal a role for orphan nuclear receptor, Nur77 in NK cell-mediated immunity against hepatocellular carcinoma (HCC). Interestingly, abundance of Nur77high NK cells was found in livers with chronic hepatitis B and viral-driven HCC. While Nur77 was previously linked to T cell exhaustion, our present study unexpectedly uncovered that Nur77 is instead associated with NK cell activation and proliferation via triggering the immunostimulatory AP-1 gene regulatory networks. Conditional ablation of Nr4a1 (encoding Nur77) in NK cells perturbed their homeostasis and accelerated tumor progression in multiple tumor models while the agonistic activation of Nur77 in NK cells ex-vivo or in-vivo enhanced their anti-tumor functions. Mechanistically, Nur77 activation attenuated CD36 expression in NK cells and conferred resistance against oxLDL-mediated immunosuppression in the TME. Collectively, our findings highlighted the potential of harnessing Nur77 agonism in improving NK cell-based immunotherapy against tumors.

Poster Abstracts

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IFNG-producing self-reactive CD4+ T cells induce autoimmune adrenalitis in a mouse model of Addison's disease

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Addison's disease (AD) is a rare but life-threatening autoimmune disease, caused by immune-mediated destruction of the adrenal cortex. Progress in therapy has been limited by the lack of mechanistic insight. Here, we establish a model of Experimental Autoimmune Adrenalitis (EAA) model that recapitulates key features of AD. In the EAA model, immunization with peptides derived from the adrenal self-antigen CYP11A1 leads to corticosterone insufficiency. We observed that the autoimmune adrenalitis is driven by IFNG produced by self-reactive CD4+ T cells, which induces the formation of granulomatous inflammation in the adrenal cortex. IFNG deficiency in CD4+ T cells largely protects the mice from adrenal dysfunction in EAA. These findings identify IFNG as a central effector in autoimmune adrenalitis and highlight IFNG pathway blockade as a promising therapeutic avenue for Addison's disease.

Poster Abstracts

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CD8 T cell differentiation biases driven by TCR-ligand affinity begin as inverse regulation of anti-viral and mitogenic processes

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The strength of T cell receptor (TCR) engagement by antigenic ligands governs the activation of a naïve T cell and the differentiation fate of its progeny. We coupled high-dimensional protein and RNA measurements with an influenza infection model and fate reporter to investigate the molecular pathways underlying this process in CD8 T cells. We found that strong TCR stimulation not only promoted short-lived effector differentiation but suppressed memory differentiation pathway diversion and altered expression of key mediators of survival and cell-cell interaction within memory precursors. This effector fate bias initially manifested as a shift away from interferon signalling and toward biosynthetic metabolism and mitogenic activity. Moreover, comparing T cells showing signs of acute in vivo TCR stimulation, we found that strong stimulation drove potent accumulation of TCR-induced transcripts, specifically downstream of ERK/MAPK signalling. These data support a mechanism by which quantitative changes in ERK/MAPK-dependent transcript abundance covert TCR affinity into differential biosynthetic activity, driving differentiation fate biases at multiple response stages.

Poster Abstracts

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Genetic variants in ZFP36L1 regulatory elements govern T cell fate and autoimmune disease susceptibility

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Formation of T cell memory is usually a critical consequence of any immune challenge to protect the organism from pathogen reencounter. In autoimmune conditions aberrant memory formation can have pathogenic self-destructive consequences as antigen persists. Stem like progenitor T cells give rise to central memory in response to acute infections and serve as a supply of effector and exhausted effector cells during chronic challenges. We have previously shown that expression of the RNA binding protein ZFP36L1 is a function of TCR affinity for antigen and is essential for selection of high affinity clones into an immune response. Here, we show that high expression of ZFP36L1 is essential to promote formation of progenitor-like T cells and formation of central memory in response to acute infections. Likewise, high levels of ZFP36L1 expression and antigen alone are sufficient for the formation of long-term surviving anergic T cells in the absence of inflammation or costimulation. Mechanistically ZFP36L1 acts as a promoter of γc-cytokine signalling while dampening the induction of terminal differentiation triggered by high affinity TCR stimulation. We identify a distal enhancer element which regulates the amounts of ZFP36L1 expressed specifically in human and mouse lymphocytes. This enhancer integrates antigen, costimulatory and inflammatory signals to scale ZFP36L1 expression. Single nucleotide variants (SNVs) within the enhancer and promoter elements of ZFP36L1 are highly associated with autoimmune disease like Type 1 Diabetes, Rheumatoid Arthritis, Lupus Erythematosus and Multiple Sclerosis. Our findings rationalize how SNVs in ZFP36L1 regulatory elements result in a break of peripheral tolerance and formation of pathogenic memory potentiating autoimmune pathology.

Poster Abstracts

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Proliferation-driven epigenetic plasticity promotes memory differentiation in human T lymphocytes

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Epigenetic remodeling is a central driver of T cell development and memory differentiation, resulting in the stable imprinting of specific T cell phenotypes in cell type–specific epigenetic landscapes. Repeated proliferation induces cumulative alterations in the epigenome, leading to epigenetic drifting and, after an extended proliferation history, epigenomic destabilization. Although proliferation and differentiation are tightly linked processes in T lymphocytes, the distinct contribution of the epigenome to each one has not been fully defined. Here, we investigate the impact of proliferation on the DNA methylome of human T lymphocytes during T cell differentiation. We demonstrate that the DNA replication speed critically determines the extend of epigenetic drifting: slow replication rates preserve epigenetic integrity and stabilize existing T cell phenotypes whereas accelerated replication speeds promote epigenomic remodeling and support differentiation into T memory states. Thus, DNA replication speed functions as a tunable regulatory switch that balances phenotype stability with memory differentiation in response to external signaling cues. Notably, during thymic T cell development, proliferation episodes are regulated in a way that supports differentiation while preventing epigenetic drifting. Together, these findings identify DNA replication speed as a key regulator of epigenetic stability and plasticity in human T cells, providing new insights into how phenotype maintenance and differentiation are coordinated at the epigenetic level.

Poster Abstracts

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Bone Marrow pDCs Deliver EV Antigens to the Thymus for T Cell Tolerance

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Plasmacytoid dendritic cells (pDCs) play a role in transporting antigens from peripheral tissues to the thymus and promote T cell tolerance through induction of regulatory T cells as well as negative selection by T cell depletion (1, 2). However, precise source of antigen and mode of its acquisition are unknown. Extracellular vesicles (EVs) play crucial roles in intercellular communication and immunity (3). Using a novel highly sensitive detection reagent for EVs (4), we showed that specifically pDCs of the bone marrow (BM) exhibit an exceptionally high uptake rate of EVs in vivo. Moreover, we found that pDCs do not degrade the phagocytosed EVs in the BM but rather preserve them for transport to the thymus. Upon arrival in the thymus, EV+ pDCs upregulate MHC II expression. Thus, we hypothesize that EVs are a major source for innocuous antigen for tolerance induction in the thymus. To confirm this, we aim to deliver specific antigen to the thymus by targeting EVs and inducing antigen specific tolerance in vivo. 1. Hadeiba, H. et al. CCR9 expression defines tolerogenic plasmacytoid dendritic cells able to suppress acute graft-versus-host disease. Nat Immunol 9, 1253–1260 (2008). 2. Fu, J. et al. CXCR4 blockade reduces the severity of murine heart allograft rejection by plasmacytoid dendritic cell-mediated immune regulation. Scientific Reports 2021 11:1 11, 23815- (2021). 3. Kalluri, R. The biology and function of extracellular vesicles in immune response and immunity. Immunity 57, 1752–1768 (2024). 4. Flaskamp, L. et al. Assessing Extracellular Vesicle Turnover In Vivo Using Highly Sensitive Phosphatidylserine-Binding Reagents. Advanced Science 12, e07624 (2025).

Poster Abstracts

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A Stem-Like Vδ1+ γδ T Cell Subset Sustains Effector Responses to Recurrent Malaria Infections Over 10 Years

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Stable expansions of γδ T cells, in particular of Vδ1+ T cells, may reflect an individual adaptation to the microbial environment and play a role in the immune response to recurring Plasmodium falciparum infections. However, the underlying mechanisms of this long-term adaptation are not well understood. In this study, we used ultra-high-throughput, combinatorial barcoding-based, single-cell RNA sequencing and single-cell αβ and γδ TCR sequencing to profile γδ T cells in a 10-year longitudinal cohort. This cohort included 96 PBMC samples collected during two annual cross-sectional blood draws and shortly after febrile malaria episodes from four Malian children (aged four to five years at enrollment) exposed to intense, seasonal malaria transmission. We found that Vδ1+ γδ T cells were enriched in all Malian subjects, contrasting with the predominant Vδ2+ T cell population observed in Western donors. Specifically, we identified a distinct, stem-like subset of Vδ1+ T cells that co-express markers of self-renewal (TCF7) and markers associated with exhaustion (TOX, PDCD1, IL10, TIGIT, and HAVCR2). This subset was present in most children living in the malaria-endemic area, as confirmed by flow cytometry. Importantly, these stem-like Vδ1+ T cells shared overlapping TCR repertoires with clonally expanded, cytotoxic Vδ1+ effector cells that mediate responses to repeated malaria infections. Longitudinal TCR tracking revealed multiple expanded Vδ1 clonotypes that persisted for over ten years alongside newly expanded clonotypes that appeared at later time points. The persistent clonotypes were largely cytotoxic but accompanied by proliferative and precursor-like states during malaria seasons. These findings suggest that durable Vδ1+ effector responses are maintained by a stem-like γδ T cell subset, which supports long-term immunity in the context of recurrent malaria infections. Together, these results imply that Vδ1+ stem-like T cells replenish the pool of Vδ1+ effector T cells, providing sustained, durable immunity to recurrent malaria infections over decades.

Poster Abstracts

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Post-transcriptional regulation of the atypical NF-κB member IκBNS in regulatory T cells

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Regulatory T (Treg) cells are essential for maintaining immune tolerance, and their development and function rely on tightly controlled gene regulatory mechanisms. We aim to explore the role of post-transcriptional gene regulation mediated by Roquin family RNA-binding proteins in Treg biology. Genetic inactivation of Roquin-1 and Roquin-2 specifically in Tregs leads to spontaneous Tfr formation and Roquin-deficient Tregs fail to suppress T cell–induced colitis, underscoring their critical role in immune regulation. To analyze physiologic regulation of Roquin-1 function through cleavage by the paracaspase MALT1, we generated and characterized MALT1-insensitive mutant mice (Roquin-1Mins/Mins). These mice exhibit reduced thymic Tregs and VAT Tregs, and show an instability phenotype marked by loss of Foxp3 expression. In CLIP analyses we have identified Nfkbid (IκBNS) as a key target of Roquin, that may be central in controlling Treg development, maintenance, and stability. Notably, conditional deletion of the Nfkbid Roquin response element (NfkbidRRE) in CD4+ T cells leads to derepression of IκBNS, resulting in increased peripheral and visceral adipose tissue Tregs, but paradoxically induces severe lung inflammation. Our preliminary data establishes Roquin proteins as pivotal regulators of Treg cell fate and function and reveal how their dysregulation can uncouple Treg expansion from effective immune tolerance.

Poster Abstracts

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STAT1 signalling shapes the function of type 2 dendritic cells in lungs of weanling mice

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Conventional dendritic cells (cDCs) are immune sentinels that orchestrate T cell responses and either promote T cell immunity or tolerance depending on signals they receive from the environment. During alveolarization of the neonatal lung cytokines imprint type 2 cDCs (cDC2s) with an enhanced capacity to drive Th2 responses, thus predisposing the organism to allergy development. Yet, concurrently, microbial colonisation of the lungs drives cDC2s to promote T cell tolerance, which protects from allergies later in life. Thus, dissecting the signals and environmental cues that shape cDC functions in early life harbours potential to guide therapeutic strategies to balance effector priming with the induction of tolerance. Here, we performed longitudinal phenotypic and transcriptional profiling of lung cDCs from birth until after weaning to the transcriptional basis underlying the phenotypic and functional heterogeneity of lung cDCs. We found that although the transcriptional identity of cDC1 and cDC2 subsets is established within hours after birth, several hallmark features of DCs are upregulated within the first week of live. Gene set enrichment analyses revealed cytokine induced transcriptional signalling pathways differentially regulated with age in cDCs. cDCs from neonates until 1 week of age showed lower signalling downstream of interferons (IFN) than cDCs at weaning (3 weeks of age) and older. Conditional deletion of Stat1 in cDCs using ItgaxCre x Stat1flox mice resulted in reduced CXCL9 production from cDC2s, but not cDC1, at 3 weeks of age. Conversely, in T cells, we observed a reduction of CXCR3+ CD4+ and CD8+ T cells with an antigen-experienced effector memory phenotype. Taken together, these data show that weaning associated changes in IFN signalling imprint the functions of lung cDC2 and that loss of Stat1 in cDCs affects T cell homeostasis. We are currently investigating weaning associated changes in IFN are caused by diet or the microbiota.

Poster Abstracts

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SLy1 regulates the IL-7Rα surface expression on thymocytes and T cells

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The adapter protein SH3 domain protein expressed in lymphocytes 1 (SLy1) is an emerging member among the family of SLy/SASH1 adapter proteins. It is exclusively expressed in lymphocytes and plays an important role for their development, survival and function. SLy1-knockout (SLy1KO) mice display a reduced number of thymocytes, indicating a crucial role of SLy1 in the development of T cells. In particular, the number of double-positive and single-positive T cells is significantly decreased in SLy1KO mice, whereas the number of double negative thymocytes is increased, compared to wildtype controls. Interestingly, surface expression of the IL-7 receptor α chain (IL-7Rα), which is important for development and survival, was shown to be decreased in thymocytes and peripheral T cells of SLy1KO mice. Under basal conditions, IL-7Rα is continuously endocytosed via clathrin-coated pits and is then recycled back to the cell surface, whilst a small fraction gets degraded and replaced by newly produced receptors. Following IL-7 stimulation, IL-7Rα is downregulated on the cell surface by a shift towards receptor degradation instead of recycling. In vivo and ex vivo experiments, in which mice or isolated thymocytes and splenocytes were treated with IL-7, revealed that in SLy1KO specimens, IL-7Rα gets comparably downregulated to controls, indicating an intact degradation process. Mass spectrometry data showed that SLy1 interacts with several proteins involved in endocytosis, as well as in the recycling pathway of IL-7Rα. Recent data indicate that the intracellular proportion of IL-7Rα is not altered between SLy1KO and control T cells, whereas IL-7Rα mRNA levels are strongly increased in SLy1KO thymocytes compared to controls. Furthermore, we could detect increased protein levels of IL-7Rα in whole spleen and thymus lysates of SLy1KO mice, as well as of Ets-1, an important transcription factor regulating the expression of IL-7Rα. Thus, we propose that SLy1 plays an important role in mediating the IL-7R processing axis and aim to identify its concrete function in this regulation mechanism.

Poster Abstracts

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Cross regulation during CNS autoimmunity

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During autoimmune reactions, tissue damage can lead to the release previously sequestered autoantigens. This diversification of epitopes can recruit naïve T cells recognizing these newly released autoantigens which is thought to worsen the disease course, in a process known as epitope spreading. However, the pool of autoreactive T cells that may be recruited via epitope spreading is tightly regulated through potent mechanism of central and peripheral tolerance. Potentially harmful autoreactive naïve T cells are deleted by negative selection or are diverted into the CD4 regulatory T cell (Treg) cell lineage. We therefore hypothesized that, in tissue-specific autoimmunity, the exposure of previously sequestered 'bystander' tissue-antigens may recruit antigen-specific Tregs that can control an autoimmune response that has been elicited against a different autoantigen. We tested this hypothesis in experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis induced using the myelin-derived peptide Myelin Oligodendrocyte Glycoprotein (MOG). While MOG is an antigen that is mostly sequestered in the central nervous system (CNS) and 'ignored' in steady state, Myelin Proteolipid Protein (PLP) is tolerized in the thymus and acts as a bystander antigen during CNS autoimmunity. We used an MHC class ll tetramer to trace a small polyclonal population of PLP9-20-specific Tregs and consistent with our hypothesis, these PLP-specific Tregs accumulated/expanded in the CNS during MOG-induced EAE. By administration of a non-immunogenic mRNA vaccine encoding for PLP9-20, we were able to expand the PLP-specific Treg population by around thirty-fold. Most importantly, administering the non-immunogenic PLP-RNA vaccine after EAE induction by MOG immunization enhanced the recruitment of PLP-specific Tregs into the CNS and attenuated the course of the disease. These findings demonstrate that epitope spreading can be tolerogenic rather than immunogenic and suggest a new therapeutic approach using tolerogenic mRNA vaccination, particularly for autoimmune diseases in which the initiating autoantigen is unknown

Poster Abstracts

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Proteogenomic analyses of allergic mast cell activation

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Mast cells are ancient immune cells with key effector functions in allergic and anaphylactic reactions. Mast cells bind circulating IgE with their FCERIA surface receptor and thereby acquire a functional antigen receptor module. As mast cells and their surface-bound IgE are long-lived, their allergic antigen reactivity reflects the current and historical IgE production. Upon allergic antigen encounter, mast cells release a large number of pre-formed and newly produced bioactive substances from their granules in a process termed degranulation. Antigen-induced IgE:FCERI proximal signaling resembles that elicited by BCR and TCR activation. However, downstream signals regulating degranulation are not well understood. We performed systematic phosphoproteomic analyses of kinetic mast cell degranulation combined with loss-of-function CRISPR screens to shed light on these processes. We uncovered the involvement of metabolic pathways, novel kinase dependencies, and evidence for a calcium-dependent feed-forward loop.

Poster Abstracts

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The impact of the tumor micromilieu on gamma delta T cell function in squamous cell carcinoma

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Recessive Dystrophic epidermolysis bullosa (RDEB) is an inherited skin disorder, characterized by mucocutaneous fragility and blister formation upon minimal trauma. Patients who suffer from RDEB, often develop highly aggressive cutaneous squamous cell carcinomas (SCC). SCC in RDEB patients develop earlier and have higher metastasis rates compared to SCC in patients without RDEB. The patho-mechanisms are still largely unknown and currently there is no effective therapy available. Recently it was shown that RDEB is not limited to cutaneous issues but includes complex endotypes marked by immune cell dysregulation and hyperinflammation. We analyzed whether the aggressive nature of SCC in RDEB patients is associated with a dysfunction in tumor immune surveillance. Gamma Delta (γδ) T cells display anti-tumor functions and the presence of tumor infiltrating γδ T cells was the most significant favorable prognostic immune population among 39 human cancer types. Whereas γδ T cell function has been studied in various skin cancer entities, their role in regulating the growth of the uniquely aggressive SCC in RDEB patients has not been investigated. We discovered that circulating γδ T cells from RDEB patients have a reduced expression of cytotoxic molecules including granzyme B and granulysin compared to those of healthy donors. Moreover, we found that tumor infiltrating γδ T cells have a heterogenous phenotype, consisting of cytotoxic but also tissue resident and wound healing γδ T cells. Furthermore, we found that RDEB patients have a locally and systemically altered cytokine milieu, that might be responsible for the phenotypical changes of γδ T cells in RDEB SCC. Future experiments will elucidate if and how this environment changes the phenotype and function γδ T cells. This study will provide insights on the anti-tumor function of γδ T cells in RDEB patients with SCC and contribute to develop therapies against the highly aggressive SCC in RDEB patients.

Poster Abstracts

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Novel encoded adjuvants for genetic vaccines

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In recent years, genetic vaccine platforms have advanced at an unprecedented pace, outperforming "conventional" immunization approaches. The advantages that make genetic vaccines the best candidate for the development of new pandemic outbreak vaccines are the ability to stimulate both humoral and cell-based immune responses, the ease of synthesis and the quick turnaround time of drug manufacturing. Despite these promising properties, delivering an encoded antigen alone often fails to generate an optimal immune response. Therefore, a deeper understanding of the mechanisms orchestrated by the immune system is essential to discover novel adjuvants that could improve the immune response to genetic vaccines, by conferring higher efficacy and durability, without compromising safety. To achieve this goal, we introduce here a synthetic immunology strategy to identify and characterize genetically encoded immune adjuvants capable of enhancing responses elicited by genetic vaccines. By integrating single-cell immune profiling with bioinformatic analysis, 80 candidate adjuvants were selected for in vivo screening by using both mRNA–lipid nanoparticle (mRNA-LNP) and adenoviral vector–based vaccine platforms, allowing us to assess their immunomodulatory activity across distinct genetic delivery systems. In particular, the co-delivery of candidate immunomodulators with the target antigen enabled coordinated spatiotemporal expression, leading to the identification of multiple immunomodulators that markedly enhance the immunogenicity of genetic vaccines targeting both cancer and infectious diseases. These findings demonstrate that genetically encoded immunomodulatory molecules can effectively improve the potency and effectiveness of vaccines delivered via both mRNA and adenoviral platforms. Moreover, by dissecting the mechanisms through which these immunomodulators act, we aim to establish a comprehensive map of the molecular circuits that orchestrate optimal adaptive immunity, ultimately accelerating the clinical translation of next-generation genetic vaccination strategies.

Poster Abstracts

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Antigen-specific immune modulation with liver-targeting nanoparticles fosters immune protective regulatory T cells to delay Type 1 Diabetes

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Type 1 Diabetes (T1D) is characterized by the loss of immune tolerance to beta-cells in the pancreas, resulting in their immune-mediated destruction. Restoring antigen-specific immune tolerance, thereby circumventing critical side effects of non-specific immunosuppression is a long-awaited goal for the prevention of T1D. We tested peptide-conjugated nanoparticles developed by Topas Therapeutics that leverage the tolerogenic capacity of liver sinusoidal endothelial cells (LSECs) to restore antigen-specific immune tolerance in T1D. Considering multiple antigens being involved in the autoantigenic response in T1D, we used nanoparticles loaded with five different T1D-relevant autoantigenic peptides (TPM-T1D). Prophylactic treatment of 8-week-old non-obese diabetic (NOD) mice with multiple i.v. injections of TPM-T1D significantly lowered the incidence of hyperglycemia at 24 weeks of age (8 weeks after the last treatment). This was accompanied by an expansion of the antigen-specific CD4+ T cell pool and significantly increased frequencies of insulin-specific regulatory T cells (Tregs) in the spleen (%Tregs of CD4+: TPM-T1D vs TP: 49,3% +/- 18,3% vs 30,3% +/- 18,6%) and a trending increase in the pancreas. These findings suggest that a combination of selected pancreatic peptides, delivered to LSECs, can induce antigen-specific Treg responses in T1D and hold up disease progression. In conclusion, this approach represents an innovative option for immune modulation to reinstate antigen-specific tolerance in autoimmune diseases, while avoiding the complications of broad immunosuppression.

Poster Abstracts

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CRISPR screening identifies the E3 ligase RNF113A as a vulnerability in lymphoma

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Recurrent malignant lymphoma remains a leading cause of cancer-related mortality. The ubiquitin–proteasome system (UPS) is frequently altered in lymphoma. To systematically identify novel UPS-dependent vulnerabilities, we conducted CRISPR/Cas9 screens in a human in vitro model of transformed germinal center B cells (GCB), generated by oncogene-driven immortalization of tonsillar GCB cells and cultured with CD40L- and IL-21-expressing follicular dendritic cell–like feeders to mimic T cell help. We conducted dropout screens targeting 776 UPS genes and identified multiple regulators critical for the fitness of transformed B-cells, including known lymphoma-associated genes such as FBXW11 and AMBRA1, as well as previously uncharacterized candidates. Among these, we focused on the RNA-binding E3 ligase RNF113A, which was previously implicated in lung cancer. Functionally, RNF113A loss sensitized lymphoma cells to therapy. Specifically, loss of RNF113A increased cell death following treatment with alkylating agents and BCL-2 inhibition, while RNF113A knockdown enhanced early apoptotic responses to both therapies. To further assess the consequences of RNF113A deficiency, we performed transcriptomic analysis, revealing broad changes in gene expression programs associated with immune signaling and survival pathways. Together, these studies identify RNF113A as a previously unrecognized regulator of GC-derived lymphoma survival and therapy response, highlighting the UPS as a source of actionable vulnerabilities in B-cell malignancies.

Poster Abstracts

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Lymph node blood endothelial cells prime alloreactive CD4+ T cells to initiate acute graft-versus-host disease

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In acute graft-versus-host disease (aGvHD), donor CD4+ T cells are activated by antigen-presenting cells (APCs) and migrate to damage target tissues. While recent studies suggested that non-hematopoietic APCs can initiate aGvHD, their identity remained unclear. During disease initiation, allogeneic T cells migrate to secondary lymphoid organs (SLOs) before infiltrating target tissues. Therefore, we investigated where and which non-hematopoietic cells first activated donor CD4+ T cells. Using murine allo-HCT models, conditional MHC class II deletion, and single-cell RNA sequencing, we found that alloreactive CD4+ T cells were activated in SLOs independently of Ccl19+ stromal cells and lymphatic endothelial cells. Instead, lymph node blood endothelial cells (BECs) expressed endogenous MHC class II and costimulatory molecules at steady state and displayed transcriptional programs consistent with antigen presentation. Following total body irradiation, BECs upregulated MHC class II, CD80, and CD86 via IL-12–driven IFNγ signaling, and IL-12 blockade ameliorated disease. Endothelial-specific MHCII deletion (MHCIIΔCdh5) reduced aGvHD severity and improved survival, whereas LEC-specific deletion (MHCIIΔProx1) was not protective, implicating BECs as key non-hematopoietic APCs. Loss of MHC class II on hematopoietic cells (MHCIIΔVav1) delayed and attenuated disease, while combined endothelial and hematopoietic deletion (MHCIIΔVav1ΔCdh5) abolished early TCR signaling and conferred marked protection. Consistently, mice expressing MHCII on endothelial but not hematopoietic or intestinal epithelial cells (B6.MHCIIΔVav1ΔVil vs. B6.MHCIIΔVav1ΔVilΔCdh5) mice exhibited poorer survival, underscoring the pathogenic role of BEC antigen presentation. Together, these findings identify LN BECs as critical non-hematopoietic APCs that initiate pathogenic CD4+ T cell responses and highlight early IL-12 blockade as a promising therapeutic strategy after allo-HCT.

Poster Abstracts

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From TCR–CD3 Architecture to Synthetic Receptors with Tunable Antigen Discrimination

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The T cell receptor (TCR) is distinguished from other immune receptors by its remarkably complex octameric architecture, comprising CD3 subunits that collectively encode ten immunoreceptor tyrosine-based activation motifs (ITAMs), along with additional conserved sequence features whose functions remain poorly understood. This extraordinary complexity has long been hypothesized to underlie the ultrasensitivity of T cells, enabling activation by as few as a single agonist peptide–MHC complex. However, how the architecture of the TCR–CD3 complex mechanistically encodes antigen discrimination remains unresolved. Here, we present a systematic dissection of the TCR–CD3 complex to define how ITAM multiplicity, ITAM identity, and non-canonical cytoplasmic motifs shape T cell responses across ligands spanning a wide range of potencies. Surprisingly, neither ITAM number nor identity influenced discriminatory power of the TCR. Instead, we identify a unique and previously unappreciated role for the CD3ζ and ε chains in modulating TCR cross-reactivity through two independent mechanisms mediated by basic-rich sequences (BRS) and proline-rich sequence (PRS). We propose that these conserved motifs regulate antigen discrimination by tuning the efficiency of CD3 chain association with Lck via BRS, and by promoting TCR association with adaptor proteins via PRS. We further demonstrate that surface TCR density is an additional factor limiting antigen discrimination by T cells. Leveraging these mechanistic insights, we engineer a synthetic T cell receptor that incorporates modified signaling domains within the native TCR framework, preserving antigen specificity while achieving comparable sensitivity to high-affinity on-target epitopes and significantly reduced responses to lower-affinity off-target epitopes. Together, our findings reveal how TCR signaling governs ligand discrimination and establish a new conceptual framework for the rational TCR design. This work enables the development of safer, more precise immunotherapies with broad applicability across antigens and cancer types.

Poster Abstracts

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Regulation of ILC differentiation and maintenance by the intestinal epithelium

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Daily confronted with microbiota, pathogens and nutrients, the intestine harbours high numbers of tissue-resident innate lymphoid cell (ILC) subsets, with the small intestinal lamina propria representing the major residence site of mature ILC3. While the fetal liver and the adult bone marrow have been proposed as the main sites of ILC development pre- and postnatally, respectively, increasing evidence indicates that ILC differentiation may additionally occur in peripheral tissues. Accordingly, we show that the fetal gut harbours not only mature ILC subsets but also immature populations such as common lymphoid and ILC-committed progenitors, strengthening the concept that the fetal intestine may represent a site of ILC-poiesis during ontogeny. Based on this data, we aimed at characterizing the niche and relevant cytokine sources promoting ILC differentiation in the intestine and show that the intestinal epithelium contributes to IL-7 production for intestinal ILC3 differentiation and maintenance postnatally.

Poster Abstracts

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Identification of novel autoantibodies for diagnosing seronegative rheumatoid arthitis

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The presence of autoantibodies is a characteristic feature of rheumatoid arthritis (RA) distinguishing this disease from other joint disorders. The hallmark antibodies (Ab) of RA are anti-citrullinated protein Ab (ACPA) which are highly specific and detectable in approximately 60% of patients, often in conjunction with rheumatoid factor (RF). ACPA/RF seropositive (sp) patients are clinically distinct from seronegative (sn) patients showing a more severe disease course and worse outcome. Although other Ab have been described to occur in sn patients most of them are not not suitable for diagnosis due to their low specificity. Therefore identification of novel Ab useful for diagnosing sn patients is still an area of intense research. To address this issue we have used in previous studies a 16k protein array presenting 6,371 human proteins for de-novo discovery of autoantibodies using sera from RA patients and appropriate controls (doi:10.3390/molecules27041452). Then 752 differentially reactive proteins were used to generate a high density array containing more than 60,000 overlapping peptides. Out of these a set of 255 differentially reactive peptides was selected for generation of a Luminex bead array and subsequently characterised on a large cohort of clinical samples (n=461), comprising sp RA, sn RA and disease controls. From these investigations 25 candidate peptides (from different proteins) emerged which were preferentially recognized by sn patients, among which 70% showed at least one reactivity as compared to 38% of disease and 24% of healthy controls. All peptides showed at least 96% specificity for sn RA while their sensitivity ranged from 2.1%-14.6%. Remarkably, the presence of multiple reactivities (directed to at least 3 unrelated peptides) was observed in 13.5% of sn RA but in less than 1% of disease controls thus proving highly specific for sn RA. Based on these promising data the bead assay will be further developed and may indeed become a useful diagnostic tool for identifying a subset of sn RA patients. The clinical relevance remains to be determined in future studies involving cohorts of well-characterized RA patients.

Poster Abstracts

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HDAC1 as a regulator of CD4+ T cell anergy and maintenance in the face of persistent cutaneous self-antigen

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Autoimmune diseases are characterized by chronic persistent presentation of self-antigens, which cannot be cleared and therefore drive ongoing immune activation and inflammation, thus leading to chronic stimulation of self-reactive CD4+ T cells. Chronic stimulation of T cells can result in T cell hyporesponsiveness and induction of anergy. Indeed, we observed that anergy is induced in response to cutaneous self-antigen in a well-established mouse model of T cell-driven experimental skin autoimmune disease (K5/TGO). In this model ovalbumin (Ova) is expressed by keratinocytes in a tetracycline-dependent manner and adoptively transferred naive Ova-specific CD4+ OTII T cells elicit skin inflammation in K5/TGO recipients. Based on the notion that histone deacetylases (HDACs) regulate the acetylation status of histones and non-histone proteins and thus HDACs control differentiation and function of CD4+ T cells, we hypothesized that HDACs also govern T cell anergy. To elucidate the role of HDAC1 we transferred HDAC1-deficient and WT OTII T cells into K5/TGO mice. HDAC1-deficient T cells induced increased skin inflammation compared to WT OTII T cells. scRNA-sequencing revealed that HDAC1 in T cells differentially regulates genes involved in immune regulation and anergy in the skin-draining lymph nodes (sdLNs). Using flow-cytometric analyses of T cells we confirmed that HDAC1-cKO OTII T cells induced lower fractions of anergic cells in the face of persistent self-antigen. This correlated with increased numbers of HDAC1-cKO effector T cells in the sdLNs. Interestingly, numbers of peripherally induced Foxp3+ regulatory T cells (pTreg) were comparable in HDAC1-cKO and WT OTII T cells recovered from K5/TGO recipients, which suggests that distinct mechanisms regulate the induction of anergy of effector T cells and pTreg.

Poster Abstracts

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B cell anti-tumor immune responses in renal cell carcinoma patients

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Renal cell carcinoma (RCC) is one of the most prevalent cancer types worldwide, in which clear cell RCC (ccRCC) is the most common subtype that accounts for over 75% of all RCC. The tumor microenvironment of ccRCC is highly infiltrated with multiple subtypes of immune cells, including a minority fraction of B lymphocytes. Notably, high infiltration of B cells is associated with poor prognosis as tumor-educated B cells play a role in promoting renal cancer metastasis. However, the existence of tertiary lymphoid structures (TLSs), enriched with B cells, has shown a positive association with prognosis in various cancer types. Moreover, tumor-infiltrating B cells exhibit the potential to produce tumor-binding antibodies, opening avenues for therapeutic interventions. In this study we aim to detect and analyze the origin, functionality and target specificity of novel tumor-reactive antibodies derived from ccRCC patients. We used single cell RNA sequencing to characterize the specific subsets of B lymphocytes and examine the B cell immunoglobulin repertoire in ccRCC tumors. Based on these transcriptomics, we cloned and generated monoclonal antibodies originating from highly mutated and clonally expanded cells. We found that class-switched plasma cells residing within the tumors can produce antibodies with tumor-binding capabilities. These findings of tumor derived monoclonal antibodies could have the potential to reveal new targets for the therapeutics and treatment of RCC patients.

Poster Abstracts

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Inhibition of pyrimidine de novo synthesis fosters Treg cells and reduces diabetes development in models of Type 1 Diabetes

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Type 1 diabetes (T1D) is an autoimmune disease characterized by the progressive loss of immune tolerance, leading to the activation of autoreactive T cells and subsequent pancreatic β-cell destruction. Therapeutic strategies to counteract this dysregulated immune activation remain under investigation, with one promising approach being the enhancement of regulatory T cell (Treg) induction to restore immune tolerance. We investigated the immunomodulatory effects of inhibiting dihydroorotate dehydrogenase (DHODH), a key enzyme in pyrimidine de novo synthesis, using the next-generation DHODH inhibitor vidofludimus calcium (Immunic Therapeutics). Under conditions of aberrant immune activation that would normally suppress Treg induction, including continuous T-cell receptor stimulation or pro-inflammatory cytokine exposure, DHODH inhibition significantly enhanced Treg induction from naïve T cells isolated from lymph nodes of non-obese diabetic (NOD) mice ($p<0.01$), indicating a direct Treg-fostering effect in vitro. To evaluate the functional relevance of these observations, we assessed the impact of vidofludimus calcium on autoimmune activation and disease progression in preclinical mouse models of T1D. Treatment significantly reduced T1D incidence in two independent models. In an accelerated model of T1D, disease incidence was markedly decreased following DHODH inhibition ($p<0.001$), accompanied by reduced T-cell activation and increased Treg frequencies. In a virus-induced model, vidofludimus calcium not only reduced disease incidence during treatment but also provided sustained protection after treatment cessation. In conclusion, restricting pyrimidine de novo synthesis through next-generation DHODH inhibition effectively suppresses autoimmune activation while enhancing Treg responses, supporting its potential as a therapeutic strategy for T1D.

Poster Abstracts

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Reconstruction of ILC development in vitro

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Innate lymphoid cells (ILCs) play a crucial role in early immune responses and maintenance of tissue homeostasis, yet their early developmental pathways remain poorly defined. The ILC progenitor population (ILCPs) has been phenotypically characterised, but the precise developmental sequence and the relationship among the phenotypically distinct ILCp subsets is not well understood. One major obstacle has been the extremely low frequency of ILCPs in tissues, which has limited both functional and transcriptomic analyses. To address this limitation, we developed a robust in vitro differentiation system that directs cultured haematopoietic stem cells toward ILCPs using a defined cytokine cocktail and stromal support. In vitro generated ILCPs are indistinguishable from to ex-vivo isolated counterparts by flow cytometry and transcriptomic analysis. scRNA-seq profiling allowed the reconstruction of the differentiation trajectory, enabling identification of key transcriptional regulators and lineage-specific gene expression dynamics. Notably, trajectory inference revealed an unexpected relationship between ILCPs, dendritic cell progenitors, and common lymphoid progenitors, challenging current developmental paradigms. In sum, our findings provide a robust foundation for further unravelling the molecular pathways that direct ILC lineage specification.

Poster Abstracts

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Inducible protein degradation reveals inflammation-dependent function of the Treg cell lineage-defining transcription factor Foxp3

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Regulatory T cells (Treg cells) are immunosuppressive CD4 T cells defined by expression of the transcription factor Foxp3. Genetic loss-of-function mutations in Foxp3 cause lethal multiorgan autoimmune inflammation resulting from defects in Treg cell development and suppressive activity. Whether Treg cells are continuously dependent on Foxp3 is still unclear. Here, we leveraged chemically induced protein degradation to show that functionally suppressive Treg cells in healthy organs can persist in the near-complete absence of Foxp3 protein for at least 10 days. Conversely, Treg cells responding to type 1 inflammation in settings of autoimmunity, viral infection, or cancer were selectively lost upon Foxp3 protein depletion. Acute degradation experiments revealed that Foxp3 acts mostly as a direct transcriptional repressor and modulates responsiveness to cytokine stimulation. This inflammation-dependent requirement for continuous Foxp3 activity enabled induction of a selective antitumor immune response upon systemic Foxp3 depletion, without causing deleterious T cell expansion in healthy organs.

Poster Abstracts

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Thymic myeloid cells are heterogenous and include a novel population of transitional dendritic cells

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Myeloid cells, including dendritic cells (DCs) and macrophages, are essential for establishing central tolerance in the thymus by promoting T cell clonal deletion and regulatory T cell (Treg) generation. Previous studies suggest that the thymic DC pool consists of plasmacytoid DC (pDC), XCR1+ DC1 and SIRPalpha+ DC2. Yet the precise origin, development, and homeostasis, particularly of DC2, remain unresolved. Using single-cell transcriptomics and lineage-defining mouse models we identify nine major populations of thymic myeloid cells and describe their lineage identities. We show that thymus SIRPalpha+ DC population contain subsets of monocyte-derived cells–DCs (moDC), and monocyte derived macrophages (moMac), that are dependent on thymic interferons (IFNs). We further demonstrate that conventional DC2 undergo intrathymic maturation regulated by CD40 signaling. Finally, we identify a novel thymic population of CX3CR1+ transitional DC (tDC) amongst SIRPalpha+ DC that represent transedothelial DCs positioned near thymic microvessels enabling presentation of blood-born antigens in the thymus. Together, these finding reveal the thymus as a niche for diverse, developmentally distinct myeloid cells and elucidate their specific requirements for development and maturation.

Poster Abstracts

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Aging-Associated Microbiota Drives Treg Dysfunction via TNF Signaling

Jefferson Leite and Ari Waisman

Aging is associated with a chronic, low-grade inflammatory state referred to as inflammaging, which contributes to impaired immune regulation and increased susceptibility to disease. While regulatory T (Treg) cells are key mediators of immune homeostasis, their role in the context of age-related inflammation remains poorly understood. Here we demonstrate that age-related changes in the microbiota promote impaired Treg cell function, resulting in the differentiation of inflammatory T cells. In agreement, we find that aged germ-free (GF) mice exhibited a more balanced immune profile, where the Treg cells are functional and pro-inflammatory mediators are reduced, suggesting that microbial exposure is essential for the establishment of inflammaging. Furthermore, we show that the use of old microbiota in young animals was sufficient to induce pro-inflammatory T cell responses and impaired mucosal Treg cell proliferation, while young microbiota restored Treg cell function in old animals. Mechanistically, we show that exposure to aged microbiota was associated with sustained TNF signaling, elevated oxidative stress, DNA damage, and increased expression of senescence markers such as γH2AX and p16 in Treg cells. These findings uncover a microbiota-TNF- dependent mechanism by which age-associated microbial dysbiosis drives Treg cell dysfunction and promotes immune aging, highlighting the therapeutic potential of microbiota-targeted strategies to restore immune homeostasis in the elderly.

Poster Abstracts

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A T cell receptor VDJδ knockin mouse reveals new NKT and IEL subpopulations of γδ T cells

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We recently described a mouse model in which a rearranged VDJδ is placed in an orthotopic manner into the TCRδ locus . In these TCRδ-KI mouse the γδ T cells in thymus and peripheral lymphatic organs are expanded 6-10 fold. Epithelial populations using Vγ5 or Vγ7 chains are unaffected in their biology in the presence of the transgenic TCRVδ6 chain. Pairing with Vγ1 massively selects a CD44+ CD24low γδT cell subpopulation already in the thymus. These γδT cells resemble Vδ6 NKT like γδ T cells described by the group of P. Pereira. The analysis of γδNKT cells at steady state and following MCMV infection reveals two distinct subpopulations, which we propose to term γδNKT1 and γδNKT2, in analogy to αβNKT1 and αβNKT2 cells. γδNKT1 cells co-expresse IFNγ and IL-4, and αβNKT2, only expresses IL-4. At late timepoints after MCMV infection (d28 p.i.), when most virus is cleared, γδNKT cells with an activated phenotype are found expanded in lung tissue. A characteristic upregulation of the immediate early genes Fos, Jun, Dusp1 and Nr4a1 suggests persisting stimulation of the γδTCR, possibly by low levels of MCMV reactivation in the latently infected lung tissue. A cluster of γδIELs characterized by high level expression of CD160 and Xcl1, low levels of CD39 and absence of granzyme expression is found strongly elevated in frequency in knockin mice. The majority of these cells express the Vγ1 TCR chain, whereas "classical" γδ IELs predominantly express Vγ7, are CD39hi, and strongly express granzyme B. Single-cell RNAseq analysis revealed clearly separated clusters, indicating a distinct γδ IEL subpopulation, with the granzyme B–negative subset also detectable at low frequency in the IEL compartment of wild-type mice. Further characterisation of the „new/unconventional" γδ IEL subpopulation in intestinal organoid co-culture systems revealed a striking proliferative capacity with concommittant upregulation of granzyme B, suggesting a precursor function under conditions of optimal IL-15 signaling and free niches in the organoids. Potential functions in the homeostasis of the intestinal epithelium will be discussed.

Poster Abstracts

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Targeting the Gut–Placenta–Brain Axis: Prenatal Postbiotic Intervention Ameliorates Autism-Like Behaviors

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Maternal immune activation (MIA) during pregnancy is a major environmental risk factor for neurodevelopmental disorders, including autism spectrum disorder (ASD). In rodent MIA models, pregnant mice was intraperitoneally injected with the synthetic double-stranded RNA polyinosinic:polycytidylic acid [poly(I:C)], a viral infection mimic, to induce ASD offspring. Previous studies have demonstrated that crosstalk between the gut–vascular barrier (GVB) and the choroid plexus–vascular barrier (PVB) contributes to MIA-induced neurodevelopmental alterations. In parallel, postbiotics derived from Lactobacillus paracasei CNCM I-5220 have been shown to restore GVB integrity under inflammation. As the placenta represents the sole interface between the maternal and fetal compartments, we investigated whether maternal postbiotic supplementation could mitigate MIA-induced impairments via the maternal–placental–fetal axis. Pregnant mice received postbiotic treatment from plug detection until delivery. We found that postbiotic administration restored poly I:C induced maternal GVB integrity, reduced systemic inflammation, improved placental transport function, and rebalanced placental immune homeostasis. Notably, postbiotic treatment did not alter the overall placental myeloid cell population, the dominant immune compartment in the placenta, but selectively normalized cDC1 and NK cell populations following poly(I:C) challenge. At embryonic day 17.5 (E17.5), both male and female fetuses from poly(I:C)-treated dams exhibited significant fetal GVB closure, which was reversed by maternal postbiotic supplementation. Postnatally, only male offspring at 11 weeks of age showed reduced autism-like repetitive behaviors and restoration of PVB integrity, whereas these protective effects were not observed in female offspring. Together, these findings indicate that maternal postbiotic supplementation represents a promising preventive strategy against MIA-induced ASD-like phenotypes, acting across generations through a coordinated gut (GVB)–placenta (immune and transport)–brain (PVB) axis.

Poster Abstracts

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Integrative Chromatin Accessibility Atlas of Rare Monogenic Immunodeficiencies Reveals Shared and Distinct Dysregulation of Immune Cell Identity

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Inborn errors of immunity (IEIs) are rare genetic diseases marked by severe immune dysfunction, with over 500 causative genes identified. This broad genetic diversity poses challenges to a purely genetic understanding of these disorders, that leaves roughly half of suspected cases – especially in antibody deficiencies – without a definitive diagnosis. Given that immune cell function is governed by complex epigenetic and transcriptional programs, we hypothesize that the epigenome functions as an "endophenotype" linking genetic causes, biological processes, and clinical manifestations of IEIs. We generated 293 chromatin accessibility profiles across six immune cell types from 68 patients representing 28 monogenic IEIs associated with antibody deficiencies, complemented by single-cell and bulk transcriptomes and reference chromatin profiles spanning fifteen B cell development stages. We uncovered a broad spectrum of IEI-specific epigenetic changes, revealing known and novel patterns of dysregulation mirrored transcriptionally. Comparing chromatin changes across genetic subtypes of ICF syndrome, we identified both subtype-specific alterations and a shared core of transcription-regulatory changes. More broadly, we found pervasive dysregulation of B cell identity, with naïve B cells from IEI patients exhibiting stage-incongruent chromatin patterns. Integration of epigenomic and transcriptomic profiles yielded a pan-disease gene regulatory network featuring modules – including NF-κB signaling and AP-1 family members – that connect genetically distinct IEIs. Our study unveils a complex chromatin regulatory landscape underlying monogenic IEIs, and demonstrates that these disorders can converge epigenetically to share common patterns of dysregulation. Such systems-level interrogation of molecular endophenotypes presents a path towards a unified molecular understanding of the rapidly expanding set of monogenic disorders, that may guide the future diagnosis and treatment of IEIs.

IN MEMORIAM
OF
HARALD VON BOEHMER

Harald von Boehmer
1942 – 2018

Curriculum Vitae

Harald von Boehmer was born on November 30, 1942 as the youngest of three. His father, Hasso von Boehmer, a lieutenant colonel in the German army, was one of the July 20th 1944 Plotters – a group of oppositionists that planned and attempted to rid Germany of Hitler. The failed attempt led to Hasso being sentenced to death and executed when Harald was just two years old. The early loss of his father remained a challenging circumstance for Harald throughout his life.

After spending his youth in his grandfather's house and graduating from high school in Freiburg, Harald went on to study Medicine in Göttingen, Freiburg and Munich, obtaining his Medical Doctorate from Ludwig Maximilian University in Munich in 1968. He subsequently received his Ph.D. from Melbourne University, Australia (1974). He joined the Basel Institute for Immunology in 1973 and remained an active member until 1996, when he became director of the Unité INSERM 373 at the René Descartes University in Paris, France. After 3 years in Paris, Harald was recruited to Harvard Medical School and the Faculty of Arts and Sciences of Harvard University, Cambridge where he established the Laboratory for Lymphocyte Biology at the Dana Farber Cancer Institute in Boston which he ran until his retirement in 2013.

In 2019 it is difficult to remember how confused immunologists were in the early 70's about T cells and what controlled their development and subsequent activation.

In the mid-1970s, Harald, together with Jonathan Sprent, showed that stable bone marrow chimerism is the result of deletion of donor cells with reactivity against recipient MHC. The presence of suppressor cells was ruled out in these studies by showing that the addition of chimera lymphocytes to normal donor-type lymphocytes did not prevent the latter from differentiating into cytotoxic lymphocytes. During the same period, Harald and Jonathan proved that in order to be stimulated by an antigen/MHC combination, the T cells had to be exposed to the MHC allele under study during development in the thymus.

In the early 1980s, the first T cell receptor (TCR) genes were found (Tak Mak and Mark Davis discovered the human and mouse TCR, respectively), and experiments transferring TCR alpha and beta genes from one T cell clone to another allowed Harald, together with Michael Steinmetz, to unequivocally conclude that the MHC-restricted specificity was encoded by a single receptor, a finding confirmed by crystallographic studies.

Curriculum Vitae

The next question Harald decided to tackle was the issue of immunological tolerance. How is it possible that T cells can respond to many different antigenic peptides, bound by MHC proteins, but do not attack self-antigens when functioning properly? Harald and his colleagues showed that self-reactive T cells are destroyed as they develop in the thymus. This was done through a very clever experiment in which mice expressing T cells with a single TCR were produced. The antigen target of these T cells was an HY-peptide expressed only in male mice. The male-specific T cells appeared as expected in the lymph nodes of female mice, but they disappeared during development in the thymus of male animals. In later years, Harald and his colleagues readdressed this issue and reported deletion of CD4+CD8+ thymocytes in the absence of TCR editing.

The TCR transgenic mice also served to answer questions related to positive selection and the matching of specificity and function. Harald's team demonstrated that the interaction between the TCR and peptide/MHC complex determines whether a thymocyte would differentiate along the CD4+CD8– T cell lineage or the CD4–CD8+ T cell lineage. They proved that there is, in fact, positive selection, as mice lacking the appropriate MHC allele to pair with the transgenic TCR failed to generate single-positive cells, and thus development was arrested at the CD4+CD8+ stage. This was then named "death by neglect" (inability to bind MHC) as opposed to "death by negative selection" which is the active induction of apoptosis in thymocytes with high affinity for self peptides or MHC. Harald summarized these findings very fittingly: "the thymus selects the useful (positive selection), destroys the harmful (negative selection), and ignores the useless (no MHC binding)".

Harald's later work continued to add basic insights to the understanding of early T cell development, including the identification of the pre-TCR and its roles in thymocyte survival, allelic exclusion, and commitment to the αβ or γδ T cell lineage. Harald and his team also studied the generation and function of regulatory T cells with the goal of utilizing these cells to prevent or interfere with unwanted immune reactions.

After his retirement, while already living here in Seefeld in Tirol, Harald wrote in his last review: "The curiosity in T cell development is still very much alive, even after retirement, but I trust that the remaining issues are in good hands of younger scientific colleagues who identify the outstanding questions and think of clever experiments to address them".

Eulogy for Harald von Boehmer

Klaus Rajewsky

Harald von Boehmer was one of my oldest and closest friends and companions who has challenged and provoked me for decades and from whom I continuously learned and profited; and on whom I could always rely. He was one of those rare people of which I knew from the first encounter that here was a connection that would last – even though we had a terrible fight at the time. So we always kept in touch: during his time at the Basel Institute of Immunology; then in Paris at the Hôpital Necker and the beautiful house in Fontainebleau; the ten years in Boston, where we were allowed to live in his home for half a year upon our arrival and subsequently had a friendly neighborly relationship with him and his family, with many joint trips to Crane Beach followed by lobster meals; and finally the time in Seefeld.

Harald was an outstanding scientist who shaped T cell immunology with visionary experiments which today are textbook knowledge, an incorruptible critical mind, and superior intelligence. I know that I am just one of many who sought and received his advice and opinion over decades, in two or three razor-sharp sentences, often highly controversial, sometimes sarcastic or with a crushing verdict, but always helpful and to the point, uncompromising in the search for truth, yet with an open, generous heart. Speaking to the non-scientists in the audience, let me stress that I am saying this in the name of many, many colleagues and scientific friends, a truly global community of eminent researchers and of course his many collaborators and students who all had a special, loving and respectful relationship with Harald and for whom he has been a central, uncompromising scientific, intellectual and human authority.

I am reading from an email I have just received from Fred Alt: "Please give my condolences and best wishes to Harald's family. I have wonderful memories of time spent with Harald (and his family) over the many decades and feel fortunate he moved to Boston so that I got to know him even better. He may be one of the most honest individuals I have ever met. If one got a compliment from him (as you and I both did every now and then) it was worth its weight in gold. I miss all of those wonderful discussions about almost everything the three of us used to have over a bottle of wine or grappa (or both) that usually went well into the night (or morning in some cases)."

Harald's loss is a turning point. For me, it comes at an age when saying farewell becomes harder and at the same time more natural. He was one of my last old, dearest friends. Together with my wife Christine, I would like to express our affection and sympathy to Monica, Lisa, Lotta and Philip and the whole family and convey our admiration for what they did for Harald in these difficult years.

July 2018, Klaus Rajewsky

Translated and slightly modified from the German

In Memoriam of Harald von Boehmer (1942–2018)

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European Journal of Immunology, 48: 1269-1270
Published: 6 August 2018

On June 24, 2018, at the age of 76, the highly creative and accomplished immunologist Harald von Boehmer passed away, the consequence of a progressive degenerative disease. He is survived by his wife Monica, and three children, Philip, Lisa and Lotta.

Harald was a larger than life person, both physically and mentally. In addition to his scientific brilliance, he was an accomplished skier and an excellent cellist who adored Pablo Casals and the music of Schumann; if time permitted, Harald and his charming wife Monica played together, he the cello and Monica the magnificent "Bösendorfer Piano Grande". Both of them loved to entertain his many colleagues, always providing outstanding meals, with excellent wine never in short supply. Harald was a skilled conversationalist who enjoyed debating scientific, as well as, countless other topics. He was particularly adept at detecting shallow and superficial ideas, and he could be harsh when dismissing rival ideas. To battle with him was always fun and educational. Possessing a talent for being almost invariably right, few if any friend or foe escaped his criticism. However, Harald was not one to harbor resentment to anyone.

In addition to other institutions, the Deutsche Forschungsgemeinschaft (DFG) relied upon his extensive knowledge. The DFG would often ask him to co-evaluate German research programs. Once Harald was convinced of a program, he would support it even against the will of his co-evaluators. In other words, Harald's decisions were spawned from his quest for truth, his independent spirit, and his commitment to science-based values.

Harald von Boehmer obtained his M.D. from the Ludwig Maximilian University in Munich (1968), and a Ph.D. from Melbourne University, Australia (1974). In Melbourne, he worked as a post-doc with Ken Shortman at the Walter and Eliza Hall Institute (WEHI). From 1973 till 1996, he became a member of the Basel Institute of Immunology, a worldwide renowned "breeding ground" and "talent incubator" for top scientists in immunology. He then moved to Paris to head the Unité INSERM 373 at the René Descartes University (1997-2000). In 1999, he accepted a Professorship for Pathology at Harvard Medical School in Boston (USA), and he became Head of the Laboratory for Lymphocyte Biology. At the end of 2012, Harald's mysterious chronic disease caused him to retire and move to Seefeld in Tirol, Austria. Thereafter, he was a guest-professor at the Institute for Immunology at the LMU in Munich.

Harald von Boehmer was a pioneer in understanding how T cells develop and function in the immune system. Following the old saying "if you want to grow palms, you have to go to places where palms can grow", he moved from Melbourne to the Basel Institute for Immunology. Together with Jonathan Sprent (also from WEHI), he analyzed tolerance to major histocompatibility complex (MHC) antigens in tetraparental bone marrow chimeric mice. Along with Michael Steinmetz, Harald achieved a major break-through in realizing that upon transfer of alpha and beta T cell receptor (TCR) genes (cloned from their H-Y specific T cell clones) a single receptor indeed executed MHC-restricted H-Y specific antigen recognition. This "breakthrough" discovery, however, was subsequently surpassed by the demonstration that clonal deletion of immature CD4+ CD8+ thymocytes is the major mechanism of central tolerance (termed negative selection), while the generation of mature, antigen-reactive T cells requires an interaction of the alpha and beta TCR with MHC antigen (termed positive selection). Today, these experiments are considered "classics" of modern immunology. Working in Paris, Harald von Boehmer's group subsequently reported on the unique role of the pre-TCR in controlling the development of alpha/beta T cells.

Naturally occurring regulatory (suppressor) T cells (Tregs) have an essential role in preventing autoimmunity, such as type 1 diabetes, and it was known that they develop in the thymus. While working at the Harvard Medical School in Boston, Harald's group realized that Tregs are also induced in the course of a peripheral immune response towards an antigen but only if homeopathic antigen doses trigger antigen reactive T cells under non-inflammatory conditions. These results led to the vision that type 1 diabetes can be prevented by Tregs generated via immunization — a new translational aspect in a career that had, until then, focused on basic immunology.

Harald von Boehmer received numerous awards including the Louis Jeantet Prize for Medicine, the Avery Landsteiner Prize (of the German Society for Immunology), the Paul Ehrlich and Ludwig Darmstädter Prize, an honorary Med- ical degree from the Technical University Munich (TUM), and, together with Klaus Rajewsky, the Kurt A. Körber Prize for European Science. He also received the Helmholtz International Fellow Award.

Even though Harald von Boehmer's "classics" (classical experiments) did not enter the Nobel path, his impact on us was immense, both as a scientist and as a person. Harald von Boehmer is no longer with us, however, thanks to his brilliant and titanic work as a scientist, his accomplishments will remain a part of the immunological paradigm — we already miss him a lot.

Harald von Boehmer 1942–2018

Iannis Aifantis & Christine Borowski
Nature Immunology, volume 19 (2018)
Published: 13 August 2018

Today, few topics in immunology receive more attention than efforts to detect, induce or reinvigorate the immune response to tumors. Although many types of immune cells affect and contribute to anti-tumor immune responses, the initial clinical findings that electrified the field focused on the T cell. Cancer biologists who grabbed the nearest immunology textbook in an effort to fully comprehend and build upon those initial clinical successes will have quickly realized that at their core, such approaches rely on understanding how T cells recognize and respond to antigen. What they may not have realized is how important Harald von Boehmer's work was in laying the foundation for this understanding.

Long before translational research was all the rage, Harald charged a segment of his lab with investigating how fundamental immunological principles might influence the onset of autoimmunity and tumor-specific immune responses. Early on he saw that breaking tolerance could result in an attack on healthy tissue or a tumor, and that suppressing the former and inducing the latter would require the study of two sides of a single coin.

Over the course of his scientific career Harald worked in or ran labs in four countries on three continents. After earning his M.D. from the Ludwig Maximilian University in Munich, Harald moved to Melbourne, Australia, where he obtained his Ph.D. under the supervision of Ken Shortman. During these early years he characterized the functions of the various cell types in the mixed-lymphocyte reaction, an assay essential for the understanding of donor–recipient compatibility in transplantation.

Shortly after receiving his Ph.D., Harald was recruited by Niels K. Jerne to the (now-defunct) Basel Institute of Immunology in Switzerland. There he worked closely with superb visiting and resident immunologists. Aided by the recent identification of genes encoding T cell antigen receptors (TCRs) and breakthroughs in transgenic technology, Harald generated mouse models that laid the foundation for understanding of the positive and negative selection of T cells, as well as T cell lineage commitment in the thymus. Through the use of these mice, he delineated the effect of major histocompatibility complex molecules and TCR cognate antigen on various stages of thymocyte development. For example, Harald demonstrated that the interaction between the TCR and peptide–major histocompatibility complex determined whether a thymocyte would differentiate along the CD4+CD8– T cell lineage or the CD4–CD8+ T cell lineage. A few years later, Harald's lab identified a previously unknown TCR, which he called the 'pre-TCR'.

In 1996, Harald left the Basel Institute of Immunology to join the Institut National de la Santé et de la Recherche Médicale and Institut Necker (Rene Descartes University) in Paris. At the Institut Necker, Harald found phenomenal immunologists, as well as direct exposure to a hospital with a tradition of the study of immunological conditions, including immunodeficiency and autoimmunity. This environment prompted him to expand the focus of his work to include more translational questions, such as those related to diabetes, T cell anergy and regulatory T cell function. However, during the same period, Harald continued to add substantial basic insights to the understanding of early T cell development; these included the identification of roles for the pre-TCR in thymocyte survival, allelic exclusion and commitment to the αβ or γδ T cell lineage.

Always fascinated by the USA, Harald had many good colleagues and friends there, and on several occasions he considered moving to a US university. In the final days of the 20th century he did, and he remained at Harvard Medical School until his retirement in 2013. Influenced by his new environment in the Smith Building of the Dana Farber Cancer Institute, Harald focused his work even more heavily on human disease. His lab made substantial contributions to the understanding of T cell leukemia, in particular the role of the Notch family of signaling receptors in this malignancy. With colleagues in the lab, Harald also published important insights into the mechanisms through which different T cell populations respond to tumors and destroy pancreatic β-cells. At the same time, he never stopped pursuing knowledge of the basic mechanisms that affect T cell development. While in Boston, his lab described mechanisms that affect the development of regulatory T cells in the thymus and the periphery and continued to publish insights into the structure and function of the pre-TCR.

After closing his Boston lab, Harald returned to his alma mater as a guest professor at the Institute for Immunology of the Ludwig Maximilian University in Munich. In his writings during this period, Harald expressed optimism about the future of immunological research. In one of his final Reviews, he mused that "The curiosity in T cell development is still very much alive even after retirement but I trust that the remaining issues are in good hands of younger scientific colleagues who identify the outstanding questions and think of clever experiments to address them" (von Boehmer, H. Front. Immunol. 5, 424 (2014)).

The immunology community would have enough to thank Harald for if the only thing he left was the enormous body of immunological knowledge he revealed. But he left more than that—he left a global network of trainees, colleagues and friends who ben-

efited from his relentless insistence on rigor, thoroughness, preparedness and creative thinking. As two of Harald's doctoral trainees, we can attest that thanks to his directness, it might not have always felt like we were benefiting while he conveyed his opinion of our work during Monday morning lab meeting. But we can also say with conviction that at the end of the day, it was always obvious that Harald's comments were made with our best interests in mind.

Harald passed away on 24 June 2018 at age of 75. His piercing intelligence, candor and unwavering support will be sorely missed.

HARALD VON BOEHMER
SELECTED PUBLICATIONS

Tolerance in T-cell-receptor transgenic mice involves deletion of nonmature CD4$^+$8$^+$ thymocytes

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The mechanism of self-tolerance is studied in T-cell-receptor transgenic mice expressing a receptor in many of their T cells for the male (H-Y) antigen in the context of class I H-2D^b MHC antigens. Autospecific T cells are deleted in male mice. The deletion affects only transgene-expressing cells with a relatively high surface-density of CD8 molecules, including nonmature CD4$^+$CD8$^+$ thymocytes, and is not caused by anti-idiotype cells.

T LYMPHOCYTES recognize antigens on the surface of other cells in the context of molecules encoded by the major histocompatibility complex (MHC)[1] by virtue of the heterodimeric T cell receptor (TRC) which is composed of α and β polypeptide chains[2,3]. In binding to its ligand, the $\alpha\beta$TCR is assisted by CD8 or CD4 accessory molecules[4,5], which presumably interact with nonpolymorphic portions of class I or class II MHC molecules respectively[6–10]. Mature T lymphocytes usually do not respond to self-MHC molecules presenting self-antigens. The question of whether the mechanism of immunological tolerance involved deletion of autospecific lymphocytes has concerned immunologists over decades[11], but no direct evidence for such a mechanism has been obtained, because the great diversity of receptors generated during lymphocyte development had made it impossible to follow individual clones of cells expressing receptors specific for self-antigens.

Recently, two groups of investigators obtained monoclonal antibodies (mAb) against the products of certain Vβ genes that are expressed with unusually high frequency on T cells specific for certain class II MHC-associated alloantigens[12–14]. Using these antibodies, Kappler *et al.* and MacDonald *et al.* were able to show that in mice expressing the relevant class II MHC-associated antigens, cells expressing the particular Vβ gene products were absent from the pool of peripheral T cells and medullary thymocytes[12–14], but were present among cortical CD4$^+$8$^+$ thymocytes[12,13]. These results can be explained by deletion of autospecific cells, but the alternative possibility that their absence is the result of a change of their phenotype caused by modulation or masking of surface molecules has not been excluded.

The development of transgenic mice offers another approach to analyse the mechanism of self-tolerance. To this end we have constructed transgenic mice expressing in a large fraction of their T cells an $\alpha\beta$TCR specific for a minor histocombatibility antigen (H-Y) present on male, but not female, cells. Fertilized eggs obtained from a cross of C57BL/6J × DBA/2J mice were injected with genomic DNA harbouring the productively rearranged TCR α and β genes isolated from the B6.2.16 cytolytic T-cell clone[15]. This clone is specific for H-Y antigen in the context of class I (H-2D^b) MHC antigen and expresses a TCR β-chain coded in part by the Vβ8.2 gene segment which can be identified by the F23.1 antibody[16].

The transgenic founder mouse 71 contained four copies of the α and two copies of the β transgenes integrated on the same chromosome[17]. It was crossed with C57L mice expressing H-2^b MHC antigens, but lacking the Vβ8 gene family. Here we show that cells with the phenotype of the B6.2.16 clone that responded to H-Y antigen were frequent in female but not in male transgenic offspring, despite the fact that peripheral T cells in animals

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Table 1 Frequency of male (H-Y) antigen-specific precursors of proliferating T cells (PT-P) among CD4$^+$8$^-$ and CD4$^-$8$^+$ T cells from normal and $\alpha\beta$TCR transgenic mice

Stimulation: spleen cells (3000R) + IL-2	CD4/CD8 phenotype	Donor of responding T cells: C57L female 1/frequency		p*	$\alpha\beta$TCR transgenic female 1/frequency		p	$\alpha\beta$TCR transgenic male 1/frequency		p
C57BL/6 female	CD4$^-$8$^+$	>25,000			>25,000			>25,000		
C57BL/6 female + Con A	CD4$^-$8$^+$	2.3	(1.6–3.3)	0.66	1.8	(1.2–2.6)	0.87	2.3	(1.3–4.0)	0.18
	CD4$^+$8$^-$	NT†			6.4	(4.5–9.1)	0.60	NT†		
C57BL/6 male	CD4$^-$8$^+$	15,985	(5,029–50,802)	0.61	6.6	(4.8–9.0)	0.67	>25,000		
	CD4$^+$8$^-$	NT†			>25,000			NT†		

Lymph node cells were stained with a mixture of anti CD4-PE and anti-CD8-FITC mabs (see Fig. 1). CD4$^+$8$^-$ and CD4$^-$CD8$^+$ T cells were separated on fluorescein activated cell sorter (FACS440, Becton Dickinson). Limiting numbers of CD4$^+$8$^-$ CD4$^-$8$^+$ T cells (24 wells per group) were cultured for 8 days together with irradiated (3,000R) spleen cells (5×10^5 cells per cell) and interleukin-w (5% v/v) of partially purified supernatant from Con A-stimulated rat spleen cells[24] without or with Con A (2.5 μg ml^{-1}). Cells were collected after addition of [^{3}H]thymidine for the last 12 h of culture and incorporated radioactivity was measured by liquid scintillation counting. Negative control cultures contained no responder cells. Frequencies were calculated as described elsewhere[25].

* Probability, p, attached to the computed χ^2 (ref. 25). † NT, not tested.

First published in: Nature 1988 Jun 23;333(6175):742-6.

of both sexes expressed both transgenes[17]. T cells in male (but not female) mice had an abnormal CD4/CD8 phenotype: over 90% of T cells in male transgenic mice were $CD4^-8^-$, or expressed only low levels of CD8 molecules, and the numbers of $CD4^+8^-$T cells were very small. The cellular composition of male thymuses revealed that this unusual phenotype of peripheral T cells was the consequence of deletion of auto-specific thymocytes expressing high levels of CD8 molecules, predominantly cortical $CD4^+8^+$ thymocytes. The deletion process spared cells expressing low levels of CD8 molecules, but affected the precursors of single positive $CD4^+8^-$ cells that were not male-specific. This latter observation provides strong evidence that double-positive $CD4^+8^+$ thymocytes contain precursors of single positive $CD4^+8^-$ and $CD4^-8^+$ T cells.

T cells in females

Lymph nodes of female transgenic mice contained normal proportions of $CD4^+8^-$ and $CD4^-8^+$ T ($Thyl^+$) cells which had normal levels of CD4 as well as CD8 accessory molecules (Fig. 1*a*, *b*, *d* and *e*). But these differed in two respects from T cells in normal mice. Firstly, as previously described for β transgenic mice[15], most of them expressed the transgenic β chain on their surface (Fig. 1*b*). Secondly, as shown by limiting dilution analysis of $CD4^-8^+$ T cells, one in six proliferated specifically in response to C57BL/6 male stimulator cells, as compared with one in 16,000 in normal C57L female mice (see Table 1). As only every second plated T cell responded to concanavalin A (Con A), we conclude that at least 30% of $CD4^-8^+$ T cells in transgenic females have a phenotype similar to that of the B6.2.16 clone. $CD4^+8^-$ T cells from transgenic mice did not show any male-specific proliferation, but did respond to Con A (Table 1).

T cells in males

As in transgenic females, lymph nodes of transgenic males contained normal proportions of $Thy1^+$ cells, and most of them expressed the transgenic β chain on their surface (Fig. 1*c*). Northern blot analysis of the α transgene revealed comparable levels of expression in T cells from female and male mice[17]. However, the CD4/CD8 phenotype of T cells in male mice was very different from that of females: 58% of $Thyl^+$ cells were $CD4^-8^-$, 35% were $CD4^-8^+$ but expressed low levels of CD8, and 7% were $CD4^+8^-$ and expressed normal amounts of CD4 (Fig. 1*f*). Limiting dilution analysis showed that one in two $CD4^-8^+$ T cells could be induced to grow by Con A. There was, however, no detectable response to male C57BL/6 stimulator cells (Table 1). Likewise, $CD4^-8^-$ and $CD4^+8^-$ T cells were unresponsive to H-Y antigen (data not shown). These results indicate that male-specific T cells with the phenotype of the B6.2.16 clone are absent from male transgenic mice and that the lack of or low level of CD8 on transgene-expressing cells precluded male-specific responses. This conclusion is supported by the observation that cytolytic activity of the B6.2.16 clone can easily be inhibited by anti-CD8 antibodies (not shown), and by CD8 gene transfection experiments which show that CD8 molecules strongly assist antigen recognition by T cells[4,5]. As shown elsewhere[17], a high proportion of $CD4^-8^-$T cells in male mice expressed both transgenes, but had their endogenous α and β genes in germline configuration. This result, and the fact that only a few T cells expressed normal amounts of CD8 in male transgenic mice, is consistent with the notion that most T cells in male mice and $CD8^+$ T cells in female mice carry transgenic $\alpha\beta$TCR on their surface. But, owing to reduced density of CD8 molecules in male mice, they are not autoreactive.

Thymocytes

The number of thymocytes was drastically lower in male ($0.5-1.6\times10^7$ per thymus) than in female ($1.0-1.6\times10^8$ per thymus) transgenic mice.

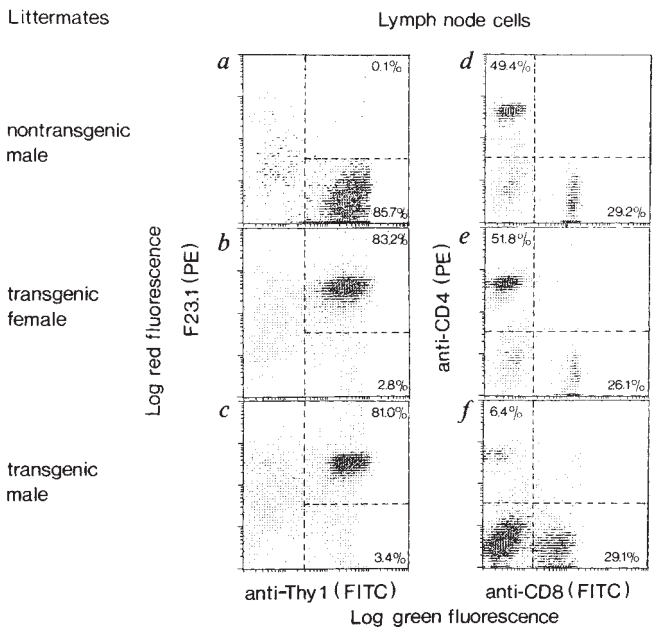

Fig. 1 Comparison of cell surface expression of $F23.1^+$ TCR β chain, CD4 and CD8 molecules on lymph node T ($Thyl^+$) cells from female and male $\alpha\beta$TCR transgenic mice and their nontransgenic male littermate as analysed by two-colour flow cytometry. Lymph node cells were stained with biotinylated F23.1 monoclonal antibody (mAb) followed by a mixture of fluorescein (FITC)-labelled anti-Thyl mab with phycoerythrin-streptavidin (PEA) (*a*, *b* and *c*) or with PE-conjugated anti-CD4 mAb followed by FITC conjugated anti-CD8 mab (*d*, *e* and *f*). In panels *a*, *b* and *c* some $Thyl^-$ cells (B cells) stain nonspecifically with F23.1 mAb due to the binding by Fc receptor. The presented data were obtained with one pair of 7-week-old $\alpha\beta$TCR transgenic female and male littermates. The same results were obtained with 3 other pairs of transgenic mice.

Methods. Single cell suspensions were prepared from lymph nodes (mesenteric, axillary, inguinal) and washed twice in RPM1-1640 and once in PBS with 5% FCS. For staining the following mAbs were used: FITC-conjugated anti-Thyl (ref. 21), biotin-conjugated F23.1 (ref. 16), PE-conjugated anti-CD4 (anti-mouse L3T4, Becton Dickinson) FITC conjugated anti-CD8 (anti-mouse Lyt2, Becton Dickinson). Biotin or FITC conjugation of mAbs was performed by standard procedures. Optimal concentrations of staining reagents were determined in preliminary experiments. All incubations and washings were done at 4 °C. Cells ($0.5-1\times10^6$) were incubated with either biotinylated F23.1 mAb (*a*, *b* and *c*) or anti-CD4-PE mAb (*d*, *e* and *f*). After 20 min, cells were washed twice and incubated again for 20 min with anti-Thyl-FITC mAb plus PEA (Becton Dickinson) (*a*, *b* and *c*) or with anti-CD8-FITC mAb (d, e and *f*). Finally, cells were washed three times in PBS 5% FCS and analysed for two-colour fluorescence on FACScan (Becton Dickinson) flow cytometer with a single Argon laser and logarithmic intensity scales using FACScan research software program (FRSP). Ten thousand viable cells were analysed in each sample. Dead cells were excluded from analysis using a combination of low-angle and sideways light scatter. The results are presented as 'density' plots, generated by analysis of processed data reduced to a 64×64 matrix with 16 levels. Percentages of stained and non-stained cells were calculated using FRSP. Markers were set against the 'density' plots of control samples which involved substitution of diluent alone for either one or both antibodies.

As shown in Fig. 2*a*, double-staining with F23.1 and CD3 antibodies demonstrated that most (>95%) thymocytes from transgenic females and males expressed the β transgene, and that the amount of TCR expression corresponded to the higher values of the normal spectrum of TCR densities observed in C57BL/6 mice.

In the thymus of transgenic females, two populations expressing different levels of TCR could be distinguished (Fig. 2*a*, middle panel). The one with relatively low TCR density included

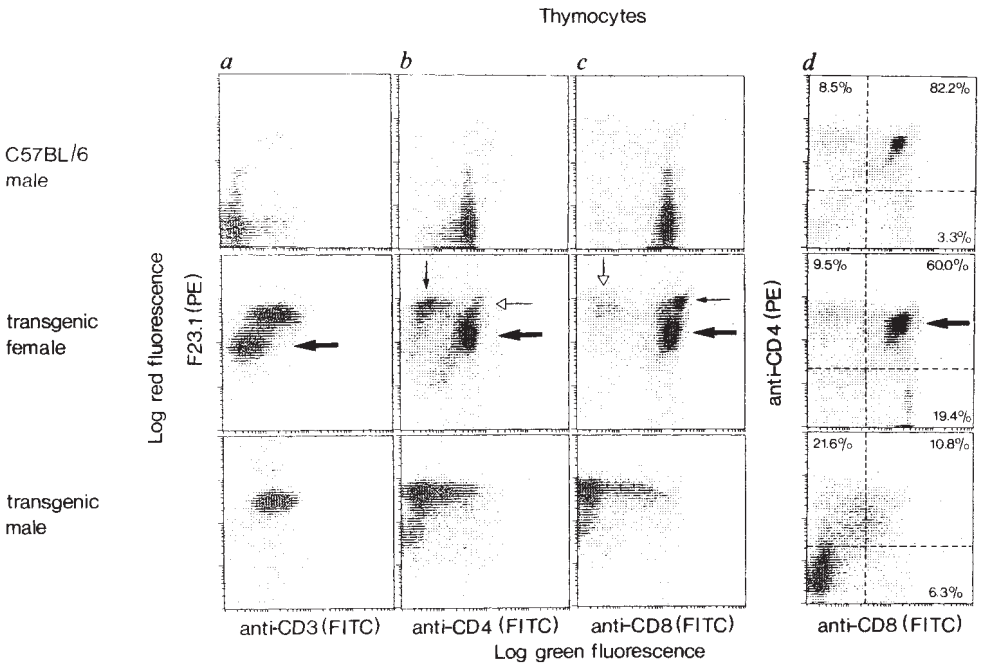

Fig. 2 Expression of F23.1$^+$ TCR β chain, CD3, CD4, and CD8 molecules on thymocyte subpopulations from normal C57BL/6 and from $\alpha\beta$TCR transgenic female and male mice. In panel *a*, cells were incubated consecutively with anti-CD3 mAb, FITC-conjugated goat anti hamster immunoglobulin, mouse immunoglobulin, biotinylated F23.1 mAb and PEA. In panels *b* and *c*, cells were stained bith biotinylated F23.1 mAb, followed by a mixture of anti-CD4-FITC (*b*) or anti-CD8-FITC (*c*) mAbs with PEA. In panel *d*, cells were stained with anti-CD4-PE followed by anti-CD8-FITC mabs. The number of cells per thymus in C57BL/6 male, $\alpha\beta$TCR transgenic female and $\alpha\beta$TCR transgenic male were: 100×10^6, 105×10^6 and 13×10^6 respectively. Thick arrows indicate the population of CD4$^+$8$^+$ thymocytes expressing a lower level of TCR, which is mostly depleted in male thymus. Open-head arrows indicate the population of CD4$^+$8$^-$, and thin arrows of CD4$^-$8$^+$ female thymocytes that express higher levels of TCR. Populations indicated by open-head thin arrows and in the upper left quadrant of middle panels *b* and *c* also contain CD4$^-$CD8$^-$ thymocytes, as indicated by virtual absence of cells in the lower left quadrant of middle panel *c*. Presence of cells in lower left quadrant of middle panel *b* is due to imperfect staining of this particular sample in this experiment. In other experiments, no F23.1$^-$CD4$^-$ cells could be seen under the same conditions.

Methods. Single thymocyte suspensions were prepared by squeezing the whole thymus through a nylon mesh into medium RPM1-1640 with 5% FCS. After washing, cells were resuspended in PBS with 5% FCS, counted and stained as indicated above with extensive washings between each step (see Fig. 1). For staining with anti-CD3, unconjugated mAb 145.2c11 (ref. 22) was used. To saturate free binding-sites of second-step reagent, cells were incubated with mouse immunoglobulin (Sigma, 1 mg ml^{-1}) for 15 min. FITC-conjugated anti-CD4 (GK1.5, ref. 23) mAb was prepared by standard procedures. Control samples were stained with each reagent alone, or in combinations omitting each single reagent. Ten thousand viable cells were analysed in each sample by FACScan flow cytometry. For details see Fig. 1.

CD4$^+$8$^+$ cells, whereas the other, expressing about tenfold more TCR, contained CD4$^+$8$^-$, CD4$^-$8$^+$ and CD4$^-$8$^-$ cells (Fig. 2*b* and *c*, middle panels).

Double-staining with CD4 and CD8 antibodies revealed significant differences between transgenic and normal C57BL/6 females with regard to the size of CD4$^-$8$^+$, CD4$^+$8$^+$ and CD4$^-$8$^-$ thymocyte subpopulations (Fig. 2*d*, upper and middle panels). The proportion of CD4$^+$8$^-$ thymocytes in transgenic females was normal, but the proportion of CD4$^-$8$^+$ thymocytes was enlarged, resulting in a reversed ratio of CD4$^+$8$^-$ to CD4$^-$8$^+$ cells as compared with normal nontransgenic mice. The proportion of CD4$^-$8$^-$ thymocytes was also noticeably higher in transgenic females than in normal mice. The increase in proportion of CD4$^-$8$^+$ and CD4$^-$8$^-$ cells was matched by a corresponding decrease in the size of the CD4$^+$8$^+$ population.

In contrast to the females, the thymus of transgenic males was severely depleted of CD4$^+$8$^+$ cells with decreased expression TCR, but contained about the same total number of CD4$^-$8$^-$ cells, which constituted the bulk of the population of male thymocytes (Fig. 2*d*, middle and lower panels). Most CD4$^-$8$^+$ cells showed low expression of CD8. Thus, the male thymus was depleted of transgene-expressing cells with relatively high levels of CD4/CD8 accessory molecules and the nonmature CD4$^+$8$^+$ thymocytes expressing decreased amounts of TCR were the main target of depletion.

Because CD4$^+$8$^+$ thymocytes are extremely steroid-sensitive, it was important to find out whether the deletion of these cells in transgenic males was a result of stress rather than of an antigen-specific deletion process. Stress in the male mice could possibly be caused by autoimmunity not detectable by *in vitro* assay. We addressed this question in reconstitution experiments using haemopoietic stem cells from transgenic (F23.1$^+$) and nontransgenic C57L (F23.1$^-$) mice (Fig. 3). T-cell-depleted bone marrow cells (BMC) from transgenic females were transferred either along or together with BMC from normal C57L females into lethally X-irradiated female and male C57L recipients. Five weeks after the transfer of the transgenic BMC, the cellular composition of the thymus in male recipients was very much like that in male transgenic mice (Fig. 3*a*, lower panel). But in the thymus of male recipients which had received a mixture of BMC from transgenic and C57L females, CD4$^+$8$^+$ thymocytes derived from F23.1$^-$ C57L donors developed normally and outgrew the transgenic F23.1$^+$ cells, which were mostly deleted (Fig. 3*b* and *c*, lower panel). On the other hand, in the female recipient, CD4$^+$8$^+$ thymocytes developed from both F23.1$^+$ and F23.1$^-$ donors (Fig. 3*b* and *c*, upper panel). Thus, because the deletion selectively affected transgene-expressing F23.1$^+$ CD4$^+$8$^+$ thymocytes in the male recipients, this experiment indicates that the deletion is a result of the interaction of autospecific thymocytes with radioresistant male cells in the thymus, and not of stress and steroid release. If the latter possibility were true, the F23.1$^-$ CD4$^+$8$^+$ thymocytes derived from C57L donors of BMC should also have been affected.

Discussion

Our study with $\alpha\beta$TCR transgenic mice provides new observations relevant to the understanding of the mechanism of self-tolerance and relevant to the clarification of the function of cortical CD4$^+$8$^+$ thymocytes in T-cell development. The drastically decreased number of thymocytes in male but not female mice is direct evidence for a deletion of autospecific cells at the level of CD4$^+$8$^+$ nonmature thymocytes. Furthermore, our results indicate that CD4 and CD8 accessory molecules are involved in the deletion process of autospecific cells.

Two questions relating to the function of double-positive

First published in: Nature 1988 Jun 23;333(6175):742-6.

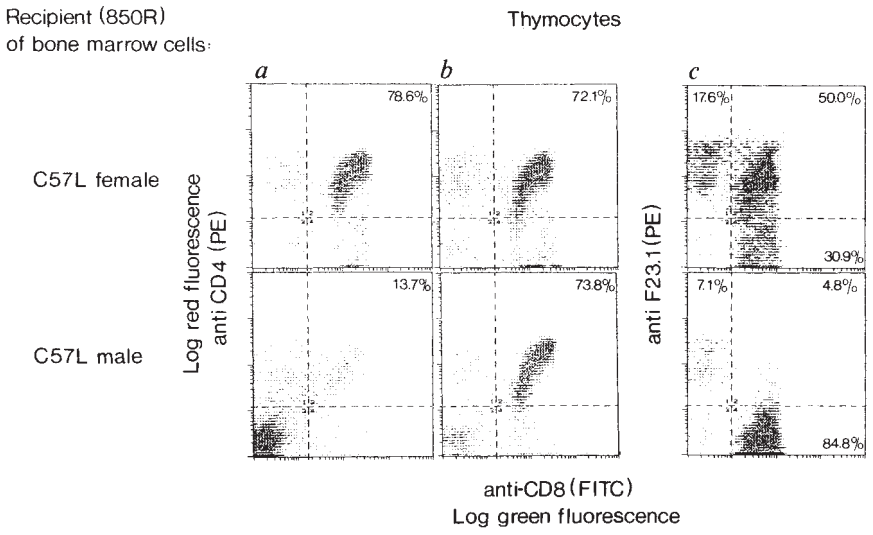

Fig. 3 Surface phenotype of thymocytes from C57L male and female irradiation chimeras reconstituted with bone marrow of $\alpha\beta$TCR transgenic female, either alone (*a*) or with normal bone marrow from C57L female (*b*, *c*). Thymocytes were stained with anti-CD4-PE (*a*, *b*) or biotinylated F23.1 (*c*) mAbs, followed by anti-CD8-FITC mAb (*a*, *b*) or a mixture of CD8-FITC mAb with PEA (*c*). In (*a*), 80% of thymocytes in the female recipient and 67% in the male recipient were stained with F23.1 mAb (data not shown).

Methods. Bone-marrow cells from transgenic or normal C57L dnor were treated with cytotoxic anti Thyl mAb (T24, ref. 21) plus rabbit complement (Cedar Lane, Ontario, Canada) for 45 min at 37 °C. After washing, 5×10^6 viable cells from the transgenic donor were injected intravenously (i.v.) into lethally irradiated (850R) eight-week-old C57L females and males, either alone or together with 0.5×10^6 viable bone marrow cells from normal female C57L. Five weeks later the mice were killed, their thymuses removed and single-cell suspensions prepared, counted and stained with anti-CD4, -CD8 and -F23.1 mAb, and analysed as described in Figs 1 and 2.

$CD4^+8^+$ thymocytes are why so many of these cells should die within the thymus[18] and whether or not they contain precursors of single positive $CD4^+8^-$ and $CD4^-8^+$ cells[19]. Our results show that the death of at least some cortical thymocytes can result from antigen-specific elimination of autoreactive cells. The deletion of nonfunctional, antigen-specific $CD4^+8^+$ thymocytes would make sense if $CD4^+8^+$ thymocytes contained precursors of functional $CD4^+8^-$ and $CD4^-8^+$ cells. Consistent with this view is our observation that $CD4^+8^-$ cells were severely depleted in male transgenic mice, despite the fact that such cells from transgenic female mice cannot be activated by male cells. An analogous finding has been reported by MacDonald *et al.*[14], who observed that $CD4^+8^-$ and $CD4^-8^+$ T cells staining with V_{β^6} antibodies were reduced to the same extent in animals positive for the *Mls*^{a}-allele of the minor lymphocyte stimulating locus (*Mls*), even though $CD4^-8^+$ from *Mls*^{a}-negative animals lacked specificity for *Mls*^{a}.

We thus favour the view that at least some double-positive $CD4^+8^+$ thymocytes act as precursors for functional single positive cells[20], even though further investigation is needed. Although we have shown that the deletion predominantly affects $CD4^+8^+$ thymocytes, we could argue that it might occur independently of accessory molecules at any stage of T-cell development. But this view is not compatible with our observation that the deletion process spares T cells that lack accessory molecules, or even T cells having low expression of CD8. Thus our experiments provide the first direct evidence that these molecules play a crucial role in the induction of tolerance. Taken together, the three observations made in male transgenic mice, namely the drastically reduced number of $CD4^+8^+$ thymocytes, the reduction of $CD4^+8^-$ cells and the occurrence of transgene-expressing cells with virtually no CD8, argue that the deletion of autospecific cells is dependent on CD4 and CD8 accessory molecules.

The results of our experiments with transgenic mice differ in at least two important aspects from others recently reported[12–14] for normal mice. Firstly, in the experiments of Kappler *et al.*[12,13], the depletion of autospecific T cells did not appear to affect $CD4^+8^+$ thymocytes. One possible reason for the difference is that different antigens are under investigation: we are looking at an antigen in the context of class I MHC antigens found throughout the cortex whereas Kappler *et al.*[12,13] are looking at an entity[21] related to class II MHC antigens which are usually not detected in the outer cortex. Thus in the latter case $CD4^+8^+$ cells can meet antigen only when reaching the cortico-medullary junction. Consequently only a minor subset of $CD4^+8^+$ cells would be deleted in the experiments of Kappler *et al.*, and this would be difficult to detect. Another possible reason for the different findings is the fact that in our transgenic mice the expression of TCR proteins is skewed towards higher levels of the range observed in $CD4^+8^+$ from normal mice. This phenomenon, as well as the increased proportion of $CD4^-8^+$ thymocytes in transgenic females, could reflect a positive selection of thymocytes by H-2^b antigens, or alternatively may be a direct consequence of expression of transgenes. We could argue therefore that the deletion of $CD4^+8^+$ thymocytes was easily detected because the majority of $CD4^+8^+$ thymocytes in transgenic mice represent a minor and more mature population of $CD4^+8^+$ thymocytes that may escape detection in normal mice, especially when representing only a fraction of cells expressing a certain idiotype. Whatever the reason for the apparent discrepancy in the results, our data indicate that the nonmature $CD4^+8^+$ populaton can be a target of deletion, whereas it is not clear whether $CD4^+8^-$ or $CD4^-8^+$ cells are susceptible to the same deletion process.

The second difference between our results and those of Kappler *et al.*[12,13] and MacDonald *et al.*[14] is that these authors did not report the presence of cells with few or no accessory molecules, spared by the deletion. Again in this case, such cells would constitute a very minor population in their experimental system, because the pool of T cells in normal mice can be easily replenished by T cells expressing different TRCs, which is not the case in transgenic mice.

As we observed normal numbers of T cells in the periphery, but not in the thymus, of transgenic males, we propose that the number of peripheral T cells can be adjusted independently of the export of newly formed cells from the thymus. This would allow the accumulation of cells with rare phenotypes in the periphery of male mice, as shown here and in the accompanying paper[17]. The fact that transgene-expressing cells with few or no accessory molecules accumulate in male mice, tends to rule out a role of anti-idiotypic cells in the deletion process; such a mechanism should eliminate transgene-expressing cells, rather than cells expressing high levels of accessory molecules.

We would like to thank Katrin Hafen, Abigail Garner and Verena Stauffer for technical assistance, Drs Werner Haas and Hung Sia Teh for critical reading and S. Kirschbaum for typing the manuscript. The Basel Institute for Immunology was founded and is supported by F. Hoffman-LaRoche & Co. Ltd, Basel, Switzerland.

Received 7 April; accepted 6 May 1988.

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First published in: Nature 1988 Jun 23;333(6175):742-6.

Thymic major histocompatibility complex antigens and the $\alpha\beta$ T-cell receptor determine the CD4/CD8 phenotype of T cells

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T-cell receptors and T-cell subsets were analysed in T-cell receptor transgenic mice expressing α and β T-cell receptor genes isolated from a male-specific, H-2D^b-restricted CD4$^-$8$^+$ T-cell clone. The results indicate that the specific interaction of the T-cell receptor on immature thymocytes with thymic major histocompatibility complex antigens determines the differentiation of CD4$^+$8$^+$ thymocytes into either CD4$^+$8$^-$ or CD4$^-$8$^+$ mature T cells.

THYMUS-derived lymphocytes (T cells) recognize antigen on the surface of antigen-presenting cells in the context of class I or class II major histocompatibility complex (MHC) molecules using the heterodimeric $\alpha\beta$ T-cell receptor (TCR)[1,2]. CD4 and CD8 molecules, expressed on the surface of T cells, bind to nonpolymorphic portions of class II and class I MHC molecules, respectively, and enhance the binding of the TCR to its ligand[3,4]. This binding of CD4 and CD8 molecules to MHC antigens may, in addition, contribute to signals leading to T-cell activation.

It is thought that the selection of the antigen-specific T-cell repertoire involves the negative selection (suppression or deletion) of autospecific T cells[5–8]. Some authors have also proposed that T cells are positively selected by thymic MHC antigens such that T cells, emerging from the thymus, bind foreign antigens predominantly in the context of self-MHC molecules[9–12]. To examine both aspects of T-cell repertoire selection we constructed TCR transgenic mice which expressed, on a large fraction of their T cells, a receptor which binds to H-Y antigen in the context of class I H-2D^b molecules. We used monoclonal antibodies that identify the transgenic receptor expressed in these mice to analyse negative selection in male $\alpha\beta$ transgenic H-2^b mice, which express the H-Y antigen as well as H-2D^b molecules. In addition, the analysis of female $\alpha\beta$ transgenic mice which express different thymic MHC antigens should reveal the possible impact of MHC molecules on the selection of T cells in the absence of nominal (H-Y) antigen.

In a previous report we have described our experiments on the mechanism of self-tolerance: from the comparison of $\alpha\beta$ transgenic male and female H-2^b mice we concluded that autospecific T cells were deleted in male mice. It was shown that this deletion involved predominantly immature CD4$^+$8$^+$ thymocytes, which contain the precursors of mature, single positive, CD4$^+$8$^-$ and CD4$^-$8$^+$ T cells[8,13].

There is less compelling evidence for the positive selection of T cells by thymic MHC antigens in the absence of nominal (H-Y) antigen: there have been reports of T cells recognizing foreign antigens predominantly in the context of those MHC molecules which they encountered during their maturation in the thymus[9–12]. It was also reported that animals that received large doses of class II MHC-antigen-specific antibodies were devoid of CD4$^+$8$^-$ T cells[14]. This could mean that antibodies can interfere with the positive selection of CD4$^+$8$^-$ T cells by thymic class II MHC antigens. These experiments do not, however, address the question of whether the $\alpha\beta$ TCR is involved in the selection process. On the basis of these and other experiments[15] one of us proposed that the interaction of the TCR on immature thymocytes with thymic MHC antigens will rescue immature T cells from programmed cell death and determine their further differentiation into mature CD4$^+$8$^-$ and CD4$^-$8$^+$ T cells. In the absence of nominal antigen, the interaction of the TCR with class II or class I thymic MHC antigens will direct the differentiation of immature T cells into CD4$^+$8$^-$ and CD4$^-$8$^+$ mature T cells, respectively[16,17]. This model predicts that in $\alpha\beta$ transgenic H-2^b mice the transgenic $\alpha\beta$ TCR should be expressed only on CD4$^-$8$^+$ and not CD4$^+$8$^-$ T cells because it was originally expressed on a class I-restricted CD4$^-$8$^+$ T cell which presumably was selected by class I MHC antigens in the thymus of C57B1/6 mice. Here we describe several observations, made in female $\alpha\beta$ transgenic mice, that are consistent with this model.

Firstly, the proportion of CD4$^-$8$^+$ thymocytes was elevated in $\alpha\beta$ transgenic H-2^b but not H-2^k or H-2^d mice. Secondly, using monoclonal antibodies specific for the transgenic receptor,

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First published in: Nature 1988 Sep 15;335(6187):229-33.

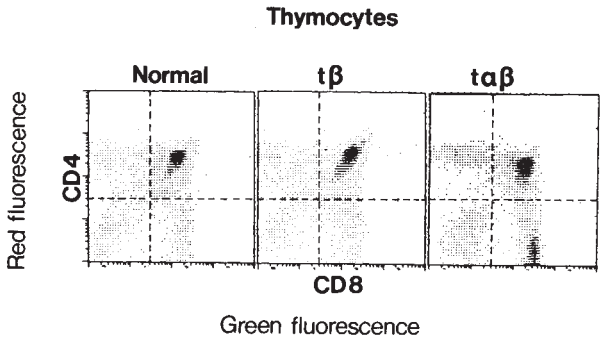

Fig. 1 The proportion of CD4/CD8 thymocyte subsets in female normal, β transgenic and $\alpha\beta$ transgenic mice. Thymocytes were stained by a mixture of phycoerythrin (PE)-conjugated anti-CD4 and fluorescein isothiocyanate (FITC)-conjugated anti-CD8 monoclonal antibodies.
Methods. Transgenic β and $\alpha\beta$ mice of the H-2^b haplotype were produced as previously described[18,13]. PE-conjugated anti-CD4 (anti-mouse L3T4, ref. 20) and FITC-conjugated anti-CD8 (anti mouse Lyt-2) were purchased from Becton Dickinson and used at a final dilution of 1 in 50. The staining of thymocytes from female C57Bl/6 (normal), transgenic β and $\alpha\beta$ mice were performed as previously described[8]. Two-colour fluorescence was analysed using a FACScan (Becton Dickinson) flow cytometer with a single Argon laser. Ten thousand viable cells were analysed in each sample. Dead cells were excluded from analysis using a combination of low-angle and sideways-light scatter. The results are presented as density plots, generated by analysis of processed data reduced to a 64×64 matrix with 16 levels. Where applicable, percentages were calculated using FACScan research software programs. The markers were set against negative controls which involved cells that were incubated in diluent alone and analysed in the same manner as the double-stained cells.

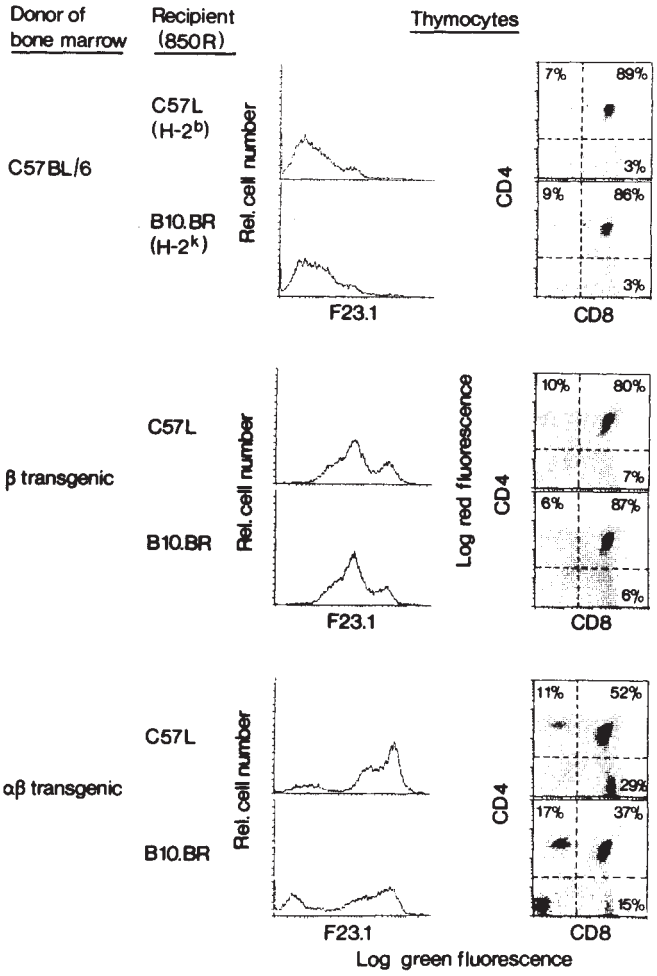

Fig. 2 The expression of transgenic β chain, CD4 and CD8 molecules on thymocytes obtained from various mice.
Methods. Female C57L and B10.BR recipient mice were lethally X-irradiated (850 rads) and reconstituted by intravenous injection of 5×10^6 anti-thy-1 treated bone marrow cells from C57Bl/6, β transgenic or $\alpha\beta$ transgenic donors. Both non-transgenic and transgenic donors of marrow cells were of the H-2^b haplotype. After six weeks the thymocytes from the recipients of marrow cells were removed, single-cell suspensions prepared and cells counted and analysed by single and double colour staining. Double staining of thymocytes with anti-CD4 and anti-CD8 antibodies was performed as described in Fig. 1. For single staining with the F23.1 antibody[21], which detects the expression of the transgenic β-chain, the thymocytes were first incubated with 10 μg ml^{-1} of F23.1 monoclonal antibody for 20 mins on ice, washed twice and then incubated with FITC-labelled sheep $(Fab')_2$ fragment anti-mouse immunoglobulin (Silenus Laboratories) at a 1 in 100 dilution for 20 mins on ice. The cells were washed three times and analysed using the FACScan flow cytometer. Five thousand viable cells were analysed in each sample.

we found that in H-2^b mice only $CD4^-8^+$ T cells expressed high levels of both the α and β transgenic TCR chains. In contrast, $CD4^+8^-$ T cells expressed high levels of the transgenic β chain only, which was usually paired with endogenous α chains. The new specificity of these receptors allowed the selection of $CD4^+8^-$ T cells by thymic class II MHC antigens. Thirdly, in $\alpha\beta$ transgenic H-2^d mice, obtained by back-crossing $\alpha\beta$ transgenic mice to DBA/2 mice, both $CD4^+8^-$ and $CD4^-8^+$ T cells expressed receptors composed of the transgenic β-chain and endogenous α-chains. The specificity of these receptors allowed the selection of $CD4^+8^-$ and $CD4^-8^+$ T cells by thymic class II and class I H-2^d MHC antigens, respectively.

T-cell subsets in transgenic mice

The proportions of thymocyte subsets classified by which of the CD4 and CD8 antigens they bear were compared in normal H-2^b and H-2^b mice that expressed either the β or $\alpha\beta$ transgenes. No significant difference was observed between C57L and β transgenic mice. In $\alpha\beta$ transgenic mice, however, the proportion of single positive $CD4^-8^+$ T cells was significantly elevated[8,13] (Fig. 1). These results indicate that the specificity of the transgenic TCR, which was originally expressed by a $CD4^-8^+$ T cell, influences the composition of thymocyte subsets. To determine whether the elevated proportion of $CD4^-8^+$ thymocytes depended on the interaction of the transgenic receptor with polymorphic domains of thymic MHC antigens, we analysed the composition of T-cell subsets in thymuses of different MHC haplotypes which had been repopulated by haemopoietic stem cells from $\alpha\beta$ transgenic, β transgenic or normal mice. In initial experiments, C57L and B10.BR recipient mice were lethally X-irradiated and repopulated with stem cells from the T-cell-depleted bone marrow of the three different donor mice. When transgenic donor cells were used, most thymocytes expressed the β transgene as detected by staining with the F23.1 monoclonal antibody showing that the repopulation was by donor cells (Fig. 2). The colonization of the H-2^b, but not the H-2^k, thymus by $\alpha\beta$ transgenic cells resulted in an elevated proportion of $CD4^-8^+$ cells compared with $CD4^+8^-$ cells. This observation was extended in a large series of repopulation experiments involving recipients expressing H-2^b MHC antigens (C57L, C57Bl/6, B10.HTG) and recipients lacking H-2^b MHC antigens (B10.BR, B10.D2, DBA/2). We consistently found that only those thymuses that expressed H-2^b MHC antigens and were repopulated by $\alpha\beta$ transgenic stem cells, had a higher proportion of $CD4^-8^+$ thymocytes. We also observed that the

First published in: Nature 1988 Sep 15;335(6187):229-33.

thymuses of MHC-mismatched (H-2^k or H-2^d), but not partly mismatched (C57Bl/6 × DBA/2)F_1 recipients, contained fewer thymocytes (10–20%) than thymuses of H-2^b animals. Furthermore, in completely allogeneic thymuses, $\alpha\beta$ transgenic stem cells yielded only one-third to one-half of the progeny of normal stem cells. Despite differences in absolute cell numbers, which may depend in part on a reaction of MHC-mismatched recipient cells towards donor cells, mice expressing H-2^b antigens in their thymus always had an elevated proportion of $CD4^-8^+$ thymocytes, and they were the only ones to do so.

We adopted an alternative approach to document the influence of thymic MHC and the specificity of the TCR on the development of thymocytes and back-crossed $\alpha\beta$ transgenic animals to DBA/2 mice (see below). In this case the thymuses of $\alpha\beta$ transgenic H-2^d/H-2^d homozygous but not $\alpha\beta$ transgenic H-2^d/H-2^b heterozygous mice contained normal numbers of thymocytes as well as a normal ratio of $CD4^+8^-$ to $CD4^-8^+$ thymocytes (3:1 to 10:1). Taken together, the results indicate that the specificity of the TCR, as well as thymic MHC antigens determine the subset composition of thymocytes.

T-cell receptors in transgenic H-2^b mice

If an interaction of the transgenic, heterodimeric TCR with thymic H-2^b MHC antigens was responsible for the elevated proportion of $CD4^-8^+$ thymocytes, most of these cells would presumably express both transgenic TCR chains. In contrast, one would expect that the interaction of TCRs with thymic class II MHC antigens, required for the selection of $CD4^+8^-$ cells, would depend on the expression of endogenous TCR chains generating TCRs with new specificities. We have previously shown that in our transgenic mice the β transgene prevents the rearrangement of endogenous V_β genes[18]. Thus, new specificities can result only from the rearrangement and expression of endogenous V_α genes, which was observed in our $\alpha\beta$ transgenic mice[13]. To investigate the expression of α-TCR genes on various T-cell subsets we prepared a monoclonal antibody that detects the transgenic α-chain. For this purpose we immunized BALB/B mice with the B6.2.16 clone from which the α and β transgenes were isolated. A B-cell hybridoma, referred to as T3.70, was obtained by fusing the immune spleen cells with the myeloma cell line AG8.653. This hybridoma produced antibodies which

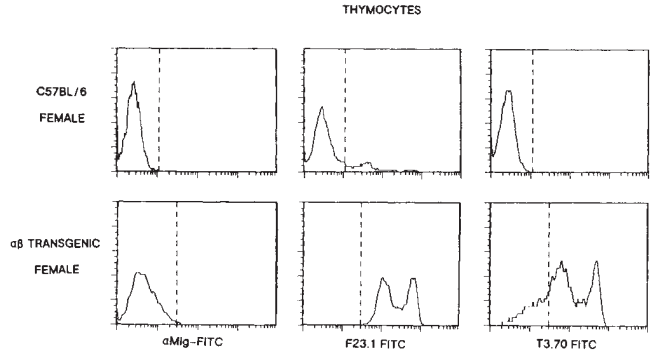

Fig. 3 Staining of thymocytes from female C57Bl/6 and $\alpha\beta$ transgenic H-2^b mice by F23.1 and T3.70.
Methods. Thymocytes from a female $\alpha\beta$ transgenic mouse were incubated with phosphate-buffered saline, F23.1 or T3.70 followed by incubation with FITC-labelled second antibody as described in Fig. 2. The stained cells were then analysed using the FACScan flow cytometer. The markers were set against thymocytes that were incubated with the FITC-labelled second antibody alone. Five thousand cells from each sample were analysed. In this experiment the percentage of thymocytes stained specifically by the F23.1 and the T3.70 monoclonal antibodies were 99.6% and 76.1%, respectively. The percentage of C57Bl/6 thymocytes stained by F23.1 and T3.70 were 10.2% and 0.0%, respectively. αMIg is anti-mouse immunoglobulin.

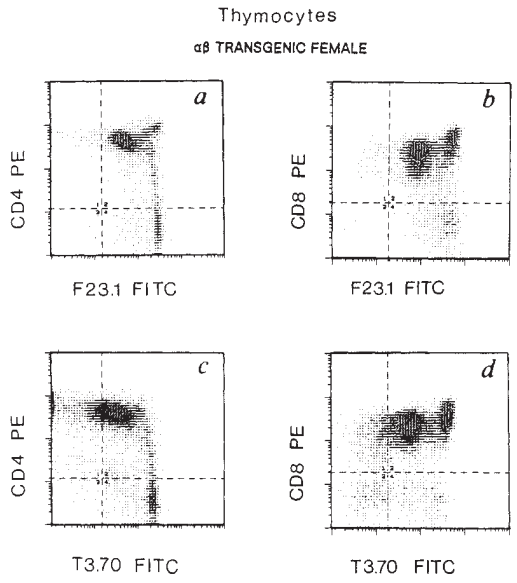

Fig. 4 Staining of thymocytes from female $\alpha\beta$ transgenic H-2^b mice by F23.1, T3.70, CD4 and CD8 antibodies. Thymocytes (yield 1×10^8) were doubled stained for F23.1 and CD4 or CD8 (*a* and *b*) and for T3.70 and CD4 or CD8 (*c* and *d*).
Methods. Thymocytes were double stained with F23.1 and anti-CD4 or anti-CD8 monoclonal antibodies by incubating the cells first with unconjugated F23.1 followed by FITC-labelled sheep $(Fab')_2$ fragment anti-mouse immunoglobulin. To saturate free mouse immunoglobulin binding sites for the FITC-labelled antibody the cells were incubated with whole mouse serum (2% v/v) for 20 mins after the FITC step. The cells were then stained directly with PE-labelled anti-L3T4 or with biotin-labelled anti-Lyt-2 followed by streptavidin phycoerythrin (both from Becton Dickinson). A similar procedure was used to stain thymocytes with T3.70 and anti-CD4 or anti-CD8. Non-specific binding of PE to the splenic T cells was minimized by washing the cells four times after incubation with the biotinylated antibody. Two-colour fluorescence was analysed as described in Fig. 1. Proportions of single positive cells expressing high levels of the F23.1 or T3.70 idiotypes were: $CD4^+$, $F23.1^+ > 95\%$; $CD8^+$, $F23.1^+ > 95\%$; $CD4^+$, $T3.70^+ < 5\%$; $CD8^+$, $T3.70^+ > 90\%$.

stained the B6.2.16 T-cell clone but not the 93.2.20 T-cell clone (derived from a β transgenic mouse), T cells from normal C57Bl/6 mice or T cells from α transgenic mice; it also precipitates a disulphide-linked heterodimer with a relative molecular mass of 90,000 (in preparation). Thus the T3.70 antibody seems to be specific for an idiotypic determinant that is dependent on the co-expression of both the α and β transgenic TCR chains.

Single staining of thymocytes from female $\alpha\beta$ transgenic H-2^b mice shows that they express low and high levels of the idiotypes recognized by either the F23.1 or T3.70 antibodies (referred to as F23.1 and T3.70 idiotypes). We already know that low receptor levels are found on $CD4^+8^+$ thymocytes. It is also clear from Fig. 3 that some thymocytes do not bear the T3.70 idiotype but are F23.1 positive, and therefore do not express the transgenic α-chain. Because α- and β-chains are present in equimolar concentrations on T cells this is consistent with our previous observation that some endogenous α genes are being expressed by T cells from $\alpha\beta$ transgenic H-2^b mice. In further experiments the differential expression of the transgenic α-chain on thymocyte subsets was analysed by double staining with one of the F23.1 or T3.70 antibodies and one of the CD4 or CD8 antibodies. From the data in Fig. 4 it is apparent the majority of $CD4^+8^+$ cells express low levels of both the T3.70 and F23.1 idiotype and therefore low levels of both α and β transgenic TCR chains (Fig. 4*a*–*d*). $CD4^+8^-$ thymocytes express high levels of the

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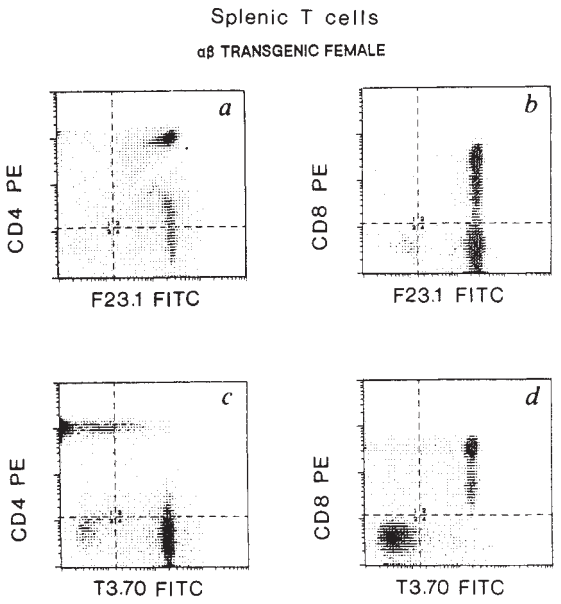

Fig. 5 Staining of peripheral, nylon-wool-purified splenic T cells from female $\alpha\beta$ transgenic H-2^b mice by F23.1, T3.70, CD4 and CD8. Nylon wool-nonadherent spleen cells from a female $\alpha\beta$ transgenic mouse were double stained with F23.1 and CD4 or CD8 (*a* and *b*) and with T3.70 and CD4 or CD8 (*c* and *d*).
Methods. Spleen cells from a female $\alpha\beta$ transgenic mouse were enriched for T cells by passing them over a nylon wool column as described[22]. The nylon wool nonadherent cells were 88.3% Thy-1^+ and 9.4% immunoglobulin-positive by single colour FACScan analyses. They were stained and analysed as described in Fig. 4. Proportions of single positive cells expressing high levels of the F23.1 or T3.70 idiotypes were: $CD4^+$, $F23.1^+ > 95\%$; $CD8^+$, $F23.1^+ > 95\%$; $CD4^+$, $T3.70^+ < 1\%$; $CD8^+$, $T3.70^+ > 90\%$.

transgenic β- but not α-chain (Fig. 4*a* and *c*). In contrast, $CD4^-8^+$ cells express high levels of both transgenic chains (Fig. 4*c* and *d*). These results indicate that the selection of $CD4^+8^-$ but not $CD4^-8^+$ cells from immature precursors requires the expression of endogenous α-chains.

The same conclusion is reached from the analysis of splenic T cells from female $\alpha\beta$ transgenic H-2^b mice (Fig. 5): again $CD4^+8^-$ T cells express high levels of the transgenic β-chain but low levels of the transgenic α-chains (Fig. 5*a* and *c*) whereas the vast majority of $CD4^-8^+$ cells clearly express high levels of both transgenic α- and β-chains (Fig. 5*b* and *d*).

T-cell receptors in transgenic H-2^d mice

Transgenic H-2^d mice were obtained by crossing $\alpha\beta$ transgenic mice with DBA/2 mice and selecting offspring that expressed α and β transgenes and were homozygous at the MHC. As the B6.2.16 clone was obtained from H-2^b mice, we expected that the transgenic TCR will not be selected in H-2^d mice because the B6.2.16 clone is not restricted by H-2^d MHC molecules. Therefore, in H-2^d mice the selection of both the $CD4^+8^-$ as well as the $CD4^-8^+$ subset should depend on the expression of endogenous α genes. The results in Figs 6 and 7 confirm this: we observe that thymocytes from five independent $\alpha\beta$ transgenic H-2^d mice (but not H-$2^d \times$ H-2^b heterozygous mice) contain more $CD4^+8^-$ than $CD4^-8^+$ single positive thymocytes (ratio 4:1, Fig. 6*a* and *b*) compared to thymocytes from $\alpha\beta$ transgenic H-2^b mice (Figs 1 and 2). Most of the $CD4^+8^+$ thymocytes in H-2^d mice express low levels of both transgenic chains similar to those observed in H-2^b mice suggesting that MHC antigens do not influence the selection of these immature cells. However, the H-2^d mice differ from the H-2^b mice in the levels of α-chain expression by single positive $CD4^+8^-$ and $CD4^-8^+$ T cells: both subsets lack high levels of the transgenic α-chain (Fig. 6*c* and *d*). This is also apparent on lymph node T cells where T cells display wide variation in the level of the T3.70 idiotype, with most cells expressing levels that are much lower (Fig. 7) than those observed on $CD4^-8^+$ T cells from transgenic H-2^b mice (Fig. 5). These data indicate that the selection of all single positive T cells in H-2^d mice requires the expression of endogenous α-chains, and that at least some of the $CD4^+8^-$ T cells in these mice can express relatively high levels of the transgenic α chain (Fig. 7*c*). This is unlike the situation in H-2^b mice because the expression of high levels of transgenic α- and β-chains in H-2^d mice does not lead to differentiation of immature $CD4^+8^+$ T cells into mature $CD4^-8^+$ T cells.

Discussion

The data reported here provide evidence that the class I MHC-restricted $\alpha\beta$ heterodimeric TCRs and thymic H-2^b MHC antigens are involved in the selection of $CD4^-8^+$ T cells. This selection occurs in the absence of the nominal (H-Y) antigen in $\alpha\beta$ transgenic female mice. The contribution of thymic MHC antigens to this selection process is evident from the fact that an elevated proportion of $CD4^-8^+$ thymocytes is observed in H-2^b, but not in H-2^k or H-2^d, thymuses repopulated by the progeny of $\alpha\beta$ transgenic stem cells. An elevated proportion of $CD4^-8^+$ cells is observed with $\alpha\beta$ transgenic stem cells but not with stem cells from β transgenic or normal C57L mice demonstrating that the $\alpha\beta$ transgenic TCR influences the selection process. $CD4^-8^+$, but not $CD4^+8^-$, cells in H-2^b mice express

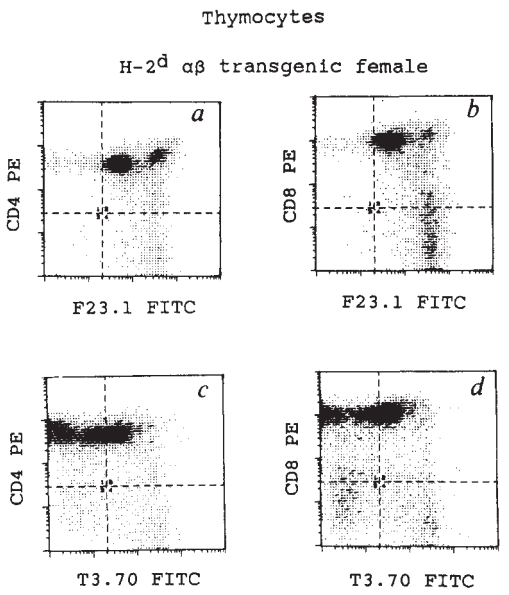

Fig. 6 Staining of thymocytes from female $\alpha\beta$ transgenic H-2^d mice by F23.1, T3.70, CD4 and CD8 antibodies. Thymocytes (yield 7×10^7) were double stained with F23.1 and CD4 or CD8 (*a* and *b*) and with T3.70 and CD4 or CD8 (*c* and *d*).
Methods. Female $\alpha\beta$ transgenic H-2^d mice were produced by backcrossing the $\alpha\beta$ transgenic founder C57B1/6 $\times$ DBA/2. (H-$2^{b/d}$)F_1 hybrid mouse with DBA/2 (H-$2^{d/d}$) mice[13]. The H-2 haplotype of the backcrosses was determined by subjecting peripheral blood lymphocytes to killing by specific antisera against K^b or K^d plus complement. Five independent $\alpha\beta$ transgenic H-2^d/H-2^d mice were analysed with similar results as shown here. Thymocytes were double stained with the indicated antibodies and analysed as described in Fig. 4. Proportions of single positive cells expressing high levels of F23.1 or T3.70 idiotypes were: $CD4^+$, $F23.1^+ > 95\%$; $CD8^+$, $F23.1^+ > 95\%$; $CD4^+$, $T3.70^+ < 5\%$; $CD8^+$, $T3.70^+ < 5\%$.

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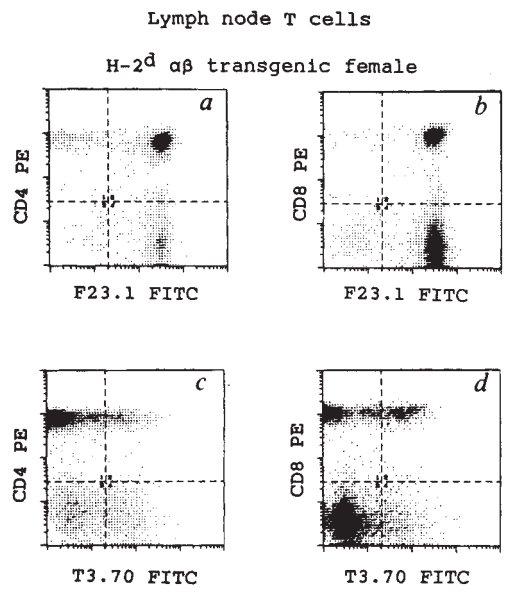

Fig. 7 Staining of lymph node cells (yield 2×10^7) from female $\alpha\beta$ transgenic H-2^d mice by F23.1, T3.70, CD4 and CD8 antibodies. **Methods.** Female $\alpha\beta$ transgenic H-$2^{d/d}$ mice were produced as described in Fig. 6. Lymph node cells were enriched for T cells by passing lymph node cells over a nylon wool column as described[22]. This preparation of nylon wool non-adherent cells contained 98.9% Thy-1^+ cells and 0.9% immunoglobulin-positive cells. The immunoglobulin-negative were double stained with F23.1 and CD4 or CD8 (*a* and *b*) and with T3.70 and CD4 or CD8 (*c* and *d*) as described in Fig. 4. Proportions of single positive cells expressing high levels of the F23.1 or T3.70 idiotypes were: $CD4^+$, $F23.1^+ > 95\%$; $CD8^+$, $T3.70^+ > 95\%$; $CD4^+$, $T3.70^+ < 10\%$; $CD8^+$, $T3.70^+ < 10\%$.

high levels of both α and β transgenic TCR chains whereas in $\alpha\beta$ transgenic H-2^d mice both subsets express lower levels of the α transgenic TCR chain confirming the importance of the $\alpha\beta$ TCR. These results are consistent with the hypothesis that the interaction of class I MHC antigens in the thymus with the $\alpha\beta$ heterodimeric T-cell receptor determines the CD4/CD8 phenotype of mature T cells in the absence of nominal antigen.

It is possible that CD4 antigens and the $\alpha\beta$ transgenic receptor are incompatible on the surface of the same cell or that CD4 molecules change the idiotype recognized by the T3.70 antibody. This does not seem likely as the majority of $CD4^+8^+$ T cells express similar levels of the determinants recognized by the F23.1 and T3.70 antibodies. In addition, this reasoning does not explain the fact that a few $CD4^+8^-$ cells in $\alpha\beta$ transgenic H-2^d mice express high levels of the T3.70 determinant although, in the same mice, most $CD4^-8^+$ cells express low levels of idiotypes recognized by the T3.70 antibody. It is also possible that the expression of the T3.70 idiotype requires CD8 molecules on the cell surface, but this is not consistent with our observation that $CD8^-$ T cells and hybridomas express the T3.70 idiotype (unpublished results) and, in general, class I-restricted T cells can express a class I-MHC-antigen restricted $\alpha\beta$ heterodimeric receptor in the absence of CD8 molecules[15]. It is possible that some interaction of the $\alpha\beta$ heterodimer with the CD8 molecule on immature $CD4^+8^+$ T cells is essential for the generation of $CD4^-8^+$ thymocytes although this would not explain the elevated proportion of $CD4^-8^+$ T cells, which express both α and β transgenic TCR chains, in H-2^b but not H-2^k or H-2^d thymuses. We therefore propose that thymic MHC antigens play an important role in the interaction of the $\alpha\beta$ heterodimer with the CD8 molecule, possibly by cross-linking the two molecules[16], which may lead to the generation of $CD4^-8^+$ T cells[16,17].

To investigate this further, we will test whether the MHC antigens needed for obtaining a high proportion of $CD4^-8^+$ cells are in fact the restricting class I H-$2D^b$ MHC antigens. It will also be important to determine whether these antigens select $CD4^-8^+$ T cells expressing high levels of both α and β transgenic TCR chains. At present, we cannot rule out the possibility that some suppression mechanism interferes with the development of $CD4^+8^-$ cells which express high levels of α and β transgenic TCR chains. We hope to investigate this possibility in $\alpha\beta$ transgenic mice which have been back-crossed to mice with severe combined immune deficiency; such mice should only express the α and β transgenes as these mice are defective in the rearrangement of endogenous TCR genes[19] and are therefore expected to be devoid of any endogenous effector T-cell population including suppressor cells.

The experiments reported here also support our earlier conclusion[8] that $CD4^+8^+$ cells contain the precursors of single positive cells. $CD4^+8^-$ T cells lack high levels of the transgenic α-chain, indicating that these T cells are not male-specific, but their numbers were significantly reduced in male $\alpha\beta$ transgenic mice[8]. The best explanation for this observation is that most of their precursors are deleted in male mice. This implies that the precursors of the $CD4^+8^-$ T cells initially express the male-specific, transgenic receptor and, later, rearrange endogenous α loci leading to the expression of new receptors selectable by class II MHC antigens. Therefore, both positive and negative selection can occur at the same stage of T-cell development, that is, negative selection by nominal self-antigen need not occur after positive selection by thymic MHC antigens. These conclusions would imply that the signals leading to positive and negative selection are different.

We thank Katrin Hafen, Beatrice Schmutz and Verena Stauffer for technical assistance and Abby Garner, Uwe Staerz and Felipe Fegueroa for providing some of the antibodies. The manuscript was typed by Shirley Kirschbaum. The Basel Institute for Immunology is supported by Hoffmann-LaRoche Co. Ltd.

Received 2 June; accepted 9 August 1988.

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Crucial role of the pre-T-cell receptor α gene in development of αβ but not γδ T cells

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In T-cell precursors, the T-cell-receptor β chain is expressed before the T-cell-receptor α chain[1,2] and is sufficient to advance T-cell development in the absence of T-cell receptor α chains[3–7]. In immature T cells, the T-cell-receptor β protein can form disulphide-linked heterodimers with the pre-T-cell-receptor α chain[8,9] and associate with signal-transducing CD3 molecules[5]. The recently cloned pre-T-cell-receptor α gene encodes a transmembrane protein that is expressed in immature but not mature T cells[9,10]. Here we show that αβ, but not γδ, cell development is severely hampered in pre-T-cell-receptor α-gene-deficient mice, which establishes a crucial role for the pre-T-cell receptor in early thymocyte development.

Intrathymic T-cell development proceeds from $CD4^-8^-$ precursors through $CD4^+8^+$ intermediates into $CD4^+8^-$ and $CD4^-8^+$ mature thymocytes[3,11]. In rearrangement-deficient mice, thymocyte development is arrested at the $CD4^-8^-3^{low}25^+$ stage[6,7,12]. Productive T-cell-receptor (TCR)-β transgenes can partly relieve the developmental block, allowing the accumulation of immature $CD4^+8^+$ thymocytes but not mature $CD4^+8^-$ and $CD4^-8^+$ T cells[3–7] that require positive selection by TCRαβ (ref. 13). In T-cell precursors the TCR-β chain forms disulphide-linked heterodimers with the pre-TCRα (pTα) chain, and can associate with signal-transducing CD3 molecules[5,8,9].

Here we report on the role of the TCRβ–pTα heterodimer in development, based on experiments with pTα-deficient mice. These animals were generated by gene targeting in embryonic stem (ES) cells using a deletion-type targeting vector. On homologous recombination, this construct eliminated exons 3 and 4 of the pTα gene encoding the connecting peptide, which contains the cysteine required for heterodimer formation, the transmembrane region, the cytoplasmic tail and most of the 3′ untranslated region (Fig. 1). Homologous recombination in ES cells and the absence of the deleted gene segment in $pT\alpha^{-/-}$ mice was verified by Southern blotting with appropriate probes (Fig. 1, and results not shown). Offspring from intercrosses of $pT\alpha^{+/-}$

First published in: Nature 1995 Jun 29;375(6534):795-8.

TABLE 1 Absolute number of thymocytes with different phenotypes

Phenotypes	$pT\alpha^+$ ($\times 10^{-6}$)	$pT\alpha^{-/-}$ ($\times 10^{-6}$)
$CD4^-8^-3^{low}25^+$	25.6	54.0
	22.0	40.4
$CD4^-8^-\delta^+$	12.4	55.1
	14.6	32.0
$CD4^+8^+\delta^+$	10.0	6.5
	14.5	11.0
$CD4^+8^+$	34.54	1.19
	34.62	1.13
$CD4^+8^+TCR\beta^{int.}$	14.50	0.24
	13.75	0.23
$CD4^+8^-TCR\beta^{high}$	3.51	0.13
	3.05	0.13
$CD4^-8^+TCR\beta^{high}$	0.81	0.09
	1.39	0.13

Numbers were obtained from two different mice of each genotype from a 5-day-old litter.

mice were killed, thymus and bone marrow removed, and single cell suspensions prepared and analysed by cytofluorometry. There were no significant differences between numbers of marrow cells from age-matched mice and thymocytes from $pT\alpha^{+/+}$ and $pT\alpha^{+/-}$ age-matched mice, whereas the number of thymocytes in pTα-deficient animals was reduced to less than 10%.

Figure 2 shows subsets from thymus of $pT\alpha^+$ and $pT\alpha^{-/-}$ mice only, because there was no difference in subsets of bone marrow and because lymphoid organs from $pT\alpha^{+/-}$ and $pT\alpha^{+/+}$ mice did not differ. Both $pT\alpha^+$ and $pT\alpha^{-/-}$ mice contain $CD4^-8^-$, $CD4^+8^+$ and single-positive $CD4^+8^-$ and $CD4^-8^+$ thymocytes, but cells with CD4 and CD8 co-receptors are proportionally under-represented in $pT\alpha^{-/-}$ mice whereas the proportion of $CD4^-8^-25^+$ cells is drastically increased (Fig. 2). Both types of mice contain CD4 and CD8 co-receptor expressing cells with low, intermediate and high levels of TCR-β chain on the cell surface. The fraction of $CD4^-8^-$ thymocytes with TCRδ chains on the cell surface was more prominent in $pT\alpha^{-/-}$ mice, and both TCRβ and TCRδ chains were stoichiometrically associated with CD3 molecules. The data also show that the TCRβ chains were associated with TCRα chains, as revealed by double-staining with TCRβ and TCR Vα2, 3.2, 8 and 11 antibodies (Fig. 2, lower right). From triple stainings with various antibodies listed in Fig. 2 and thymocyte numbers, we calculated the absolute number of cells belonging to various thymocyte subsets as shown in Table 1; the data show that the pre-TCR is not required to generate normal numbers of $CD4^-8^-3^{low}25^+$ precursors of αβ T cells. In fact, their number is increased in $pT\alpha^{-/-}$ mice, probably because of the developmental arrest in these mice (see below). There is likewise no decrease in the number of $CD4^-8^-$ or CD4 and CD8 co-receptor expressing γδ T cells (Table 1). Table 2 shows that among $CD4^-8^-3^{low}$ cells, $pT\alpha^{-/-}$ mice have normal or increased numbers of $CD44^+25^-$, $CD44^+25^+$ and $CD44^-25^+$ cells, but no $CD44^-25^-$ cells, which represent 45% in $pT\alpha^+$ mice. As most thymocyte expansion occurs in this subset[11], it is not surprising that the absolute number of $CD4^+8^+$ cells as well as mature TCR $\alpha\beta^{high}$ $CD4^+8^-$ and $CD4^-8^+$ cells is much lower in $pT\alpha^{-/-}$ than in $pT\alpha^+$ mice. Also, the proportion of TCR-αβ-positive cells among $CD4^+8^+$ cells is lower in $pT\alpha^{-/-}$ than in $pT\alpha^+$ mice. Lymph-node cells from 28-day-old $pT\alpha^{-/-}$ mice contain mature single positive CD4 and CD8 cells representing about 5% of numbers found in $pT\alpha^+$ littermates (results not shown).

TABLE 2 Subsets among $CD4^-8^-3^{low}$ thymocytes

Phenotypes	$pT\alpha^+$ (%)	$pT\alpha^{-/-}$ (%)
$CD44^+25^-$	12.1	11.0
$CD44^+25^+$	2.20	2.30
$CD44^-25^+$	40.7	86.6
$CD44^-25^-$	45.0	0.10

Percentages were calculated from the same litter as described in Fig. 2 and Table 1.

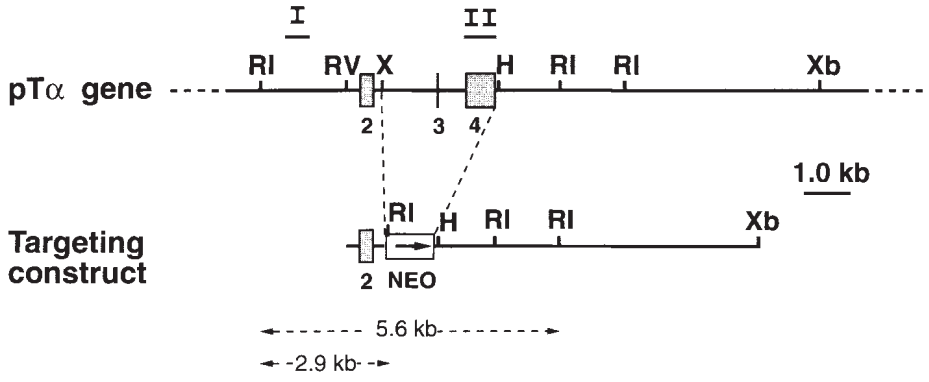

FIG. 1 Disruption of the pTα gene by homologous recombination: partial organization of the pTα locus[22] and structure of the targeting vector. The pTα gene was cloned from a 129/Ola-derived genomic library (kind gift of A. Berns, Amsterdam). The targeting vector was constructed by replacing approximately 2.6 kilobases (kb) of the pTα sequence between the unique *Xho*I site in intron 2 and a *Bgl*II site ~60 base pairs (bp) upstream of the AATAAATAAA polyadenylation site with a 1.2-kb *Xho*I–*Bam*HI fragment of pMC1neopA (Stratagene) carrying the neomycin resistance gene (*neo*). The isogenic targeting construct was electroporated into E14.1 embryonic stem cells as described[18]. ES colonies surviving G418 selection were analysed by the polymerase chain reaction (PCR) in pools of 12 using primers specific for the *neo* cassette within the *tk* promotor (ATTCGCCAATGACAAGACGCTGC) and for the pTα gene just upstream of the *Eco*RV site (GTTGGATGTTATTGGTTACTACTCCTGA), respectively. Colonies within positive pools were re-screened individually by PCR and eventually by Southern analysis using *Eco*RI-digested DNA and probe I (a 470-bp PCR fragment specific for pTα sequences outside the targeting construct, ~1.3 kb upstream of exon 2 (primers: TAGGTTTGAACTCAGAT; TGATTTCTCTCTGTAGC)). Out of ~1,600 colonies screened, 3 had undergone homologous recombination, and one of these clones (pT355) gave rise to chimaeric mice. Chimaeric males were backcrossed with $(C57BL/6 \times DBA/2)F_1$ females and heterozygous offspring carrying a mutant pTα allele were intercrossed to obtain mice deficient in pTα. The absence of the deleted pTα sequences in homozygous knockout mice was confirmed by Southern blotting of *Eco*RI-digested genomic tail DNA and hybridization with a 310-bp *Apa*I/*Bsp*EI complementary DNA fragment spanning the region of exon 4 that encodes the transmembrane portion, the cytoplasmic tail and 120 nucleotides of 3′ untranslated sequence. Abbreviations for restriction sites: RI, *Eco*RI; RV, *Eco*RV; X, *Xho*I; H, *Hin*dIII; Xb, *Xba*I.

First published in: Nature 1995 Jun 29;375(6534):795-8.

The above data indicate that pTα has no role in the development of most γδ T cells for one of three reasons: (1) because it is not expressed in the γδ lineage; (2) it cannot pair with the γ chain; or (3) because there is no need for a putative TCRγ–pTα heterodimer in γδ T-cell development. Although this issue requires further investigation, we have been unable to detect RNA for pTα in thymocytes of TCRδ surface-positive thymocytes[10]. The increase in the number of γδ cells could depend on the availability of space, and/or the possibility that ongoing γδ rearrangement is not terminated by the pre-TCR. The fact that a few TCRαβ-positive CD4/8-co-receptor-expressing thymocytes can be generated in the absence of the pre-TCR is consistent with an earlier finding of TCRα rearrangements in $TCR\beta^{-/-}$ mice deficient in pre-T-cell-receptors[6]. The authors argued that $CD4^+,8^+$ cells in $TCR\beta^{-/-}$ mice (which are often claimed erroneously not to contain $CD4^+8^+$ cells) could be of the δ lineage because they were absent in $TCR\beta^{-/-} \times TCR\delta^{-/-}$ mice. We find that in $pT\alpha^{-/-}$ mice $CD4^-8^-3^{low}25^+$ precursors of αβ T cells can differentiate, albeit inefficiently, into $CD4^+8^+$ cells with TCRαβ on the surface. We therefore propose that the pre-TCR is sufficient and necessary for the generation of $CD4^+8^+$ precursors when no other TCR-expressing cells are present, and that in the presence of TCR-positive cells, the pre-TCR is required for the transition of $CD4^+8^+25^+$ αβ T-cell precursors, through rapidly dividing $CD4^-8^-25^-$ cells into TCRαβ-expressing $CD4^+8^+$ thymocytes.

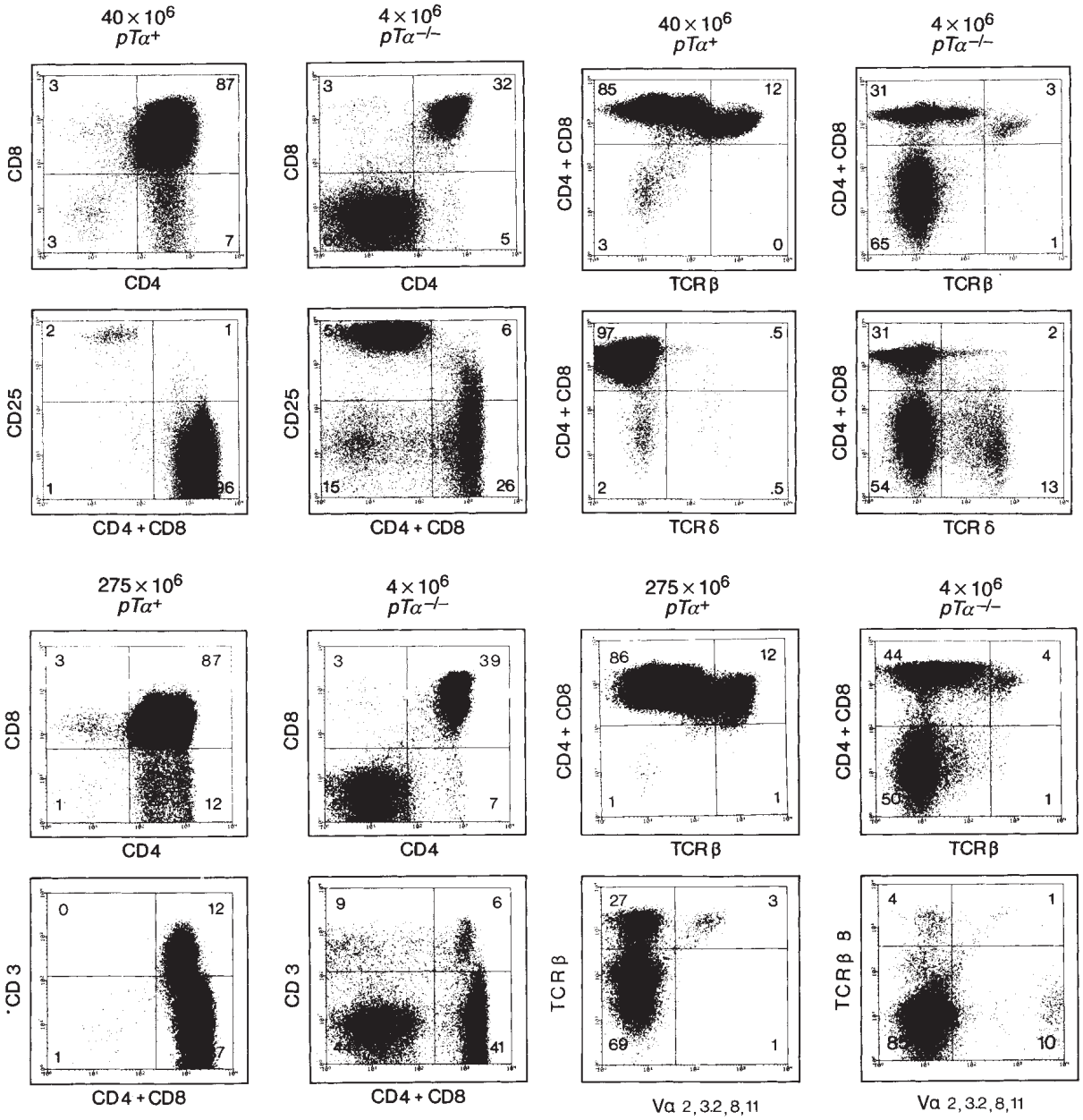

FIG. 2 Thymocyte subsets from $pT\alpha^+$ and $pT\alpha^{-/-}$ mice. Staining of thymocytes from 5-day-old (top two rows) and 28-day-old (bottom two rows) litters with CD4, CD8, TCRβ, TCRδ and TCR-V_α antibodies. All stainings were three-colour stainings with the following antibody-conjugates: anti-CD3–biotin and anti-CD3–FITC conjugates (500 A2 (ref. 19)), anti-TCRβ–biotin and anti-TCRβ–FITC (H57 (ref. 20)), anti-CD4–phycoerythrin (H129.19 Gibco), anti-CD8–biotin and anti-CD8–phycoerythrin (53-6.7 Pharmingen, San Diego), Anti-CD25–biotin (Pharmingen, San Diego), anti-CD44–phycoerythrin (ATCC collection KM81), anti-HSA–biotin (M1169 (ref. 21)), and anti-TCRδ–FITC (G3, Pharmingen, San Diego). A cocktail of TCR-V_α antibodies, namely anti-V_α2 (B20.1),anti-V_α3.2 (RR3.16), anti-V_α8 (KT50) and anti-V_α11 (RR8.1) was obtained from D. Mathis, Strasbourg. The biotin conjugates were revealed by streptavidin–Tricolor, (Caltag, San Francisco). For each of the three colour stainings ~4×10^5 cells were incubated with various reagents. In each case, 10^5 events were acquired for fluorescence-activated cell sorting by FACScan. The bright staining in the thymus (lower right) is due to thymic B cells that stain brightly with sheep anti-mouse Ig–FITC reagent, which was used to reveal the V_α antibodies.

First published in: Nature 1995 Jun 29;375(6534):795-8.

This view is consistent with data showing that TCR-positive thymocytes can induce the development of $CD4^+8^+$ cells when injected into rearrangement-deficient mice[14,15] and explains the absence of $CD4^+8^+$ thymocytes in rearrangement-deficient[6,7], TCR-negative[6] as well as CD3-negative mice (B. Malissen, personal communication). Differentiation through the $CD4^-8^-25^-$ subset requires cell-autonomous signals delivered by the pre-TCR, and for that reason in normal mice almost all $CD4^+8^+$ T cells contain productive TCR-β genes[16], whereas in the absence of the pre-TCR, only ~2% of TCRαβ-positive $CD4^+8^+$ precursors are generated by some aberrant differentiation. Nevertheless, positive selection will operate on these cells and generate mature αβ T cells whose number is regulated by homeostasis independent of the pre-TCR[17]. □

Received 12 May; accepted 8 June 1995.

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ACKNOWLEDGEMENTS. We thank U. Müller for injection and implantation of blastocysts; C. Laplace and K. Hafen for technical assistance and C. Geiger for typing; D. Mathis and C. Benoist (Strasbourg, France) for the gift of TCR-V_α antibodies, and K. Karjaleinen (Basel, Switzerland) for critical reading of the manuscript. The Basel Institute for Immunology is supported by F. Hoffmann–La Roche Ltd, Basel. The work was also supported by INSERM through the Unité INSERM 373. H.V.B. is supported by the Human Frontier Science Program Organization.

First published in: Nature 1995 Jun 29;375(6534):795-8.

Imprint

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