

Division of Immunobiology

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Our long-term research interest is to understand molecular mechanisms that regulate the development and function of immune cells. The research in our laboratory focuses on studies to investigate epigenetic and transcriptional control mechanisms that regulate CD8 coreceptor expression, on the identification of genes that control developmental processes such as CD4/CD8 cell fate decision during T cell differentiation, and on studies to reveal the various roles of Tec family kinases in different cell lineages of the immune system. The experimental strategies to address our research interests include biochemical and molecular approaches, retroviral-mediated gene transduction into hematopoietic stem cel-

MOLECULAR MECHANISMS OF T CELL DEVELOPMENT

We are interested to understand how the CD4/CD8 cell fate decision of double-positive (DP) thymocytes into helper or cytotoxic T cells is regulated. The transcriptional regulation of the Cd4 and Cd8 genes is tightly linked to the functional program of T cells. It is conceivable that factors that regulate CD4 and CD8 expression are also involved in directing DP thymocytes towards the helper or cytotoxic lineage, respectively. Therefore, it is important to understand how the coreceptor genes are transcriptionally regulated during T cell development and to identify cis-regulatory elements and trans-acting factors involved in their regulation. This will not only provide insights into transcriptional control mechanism in T cells, but may also lead to the identification of molecular factors which are involved in cell fate specifications during T cell development.

Ongoing studies in the laboratory aim to further characterize Cd8 cis-regulatory elements by performing (combinatorial) enhancer knockout experiments, to isolate additional Cd8 enhancer binding factors, and to study in detail the role of the BTB zinc finger transcription factors MAZR and PLZF during T cell development (Ref. 1). Moreover, we are investigating the role of members of the histone deacetylase family in T cells.

Transcriptional control of Cd8 gene expression during T cell development

Our laboratory is studying the regulation of CD8 coreceptor expression, a key molecule in the immune system for the development of the cytotoxic T cell lineage. CD8 coreceptor expression is tightly regulated during thymocyte development by the activity of at least five different cis-regulatory elements. We recently linked Cd8 enhancer function with chromatin remodeling of the adjacent genes Cd8a and Cd8b1 (Cd8) and demonstrated epigenetic control of the Cd8 gene complex (Bilic et al., 2006, Nature Immunology, 7, 392).

We further aim to identify Cd8 enhancer binding factors. Since important Cd8 cis-regulatory elements are expected to be evolutionary conserved, a cross-species comparison of the Cd8a and Cd8b genomic loci to search for evolutionary conserved regions (ECR) was performed. This approach revealed several ECRs ranging from 200bp to 500bp within known Cd8 enhancers, which can be used as molecular baits for the isolation of binding factors. Moreover, this approach led to the identification of a novel evolutionary conserved Cd8 enhancer element that is currently analyzed in more detail. Our results demonstrate that a combination of bioinformatic and biological approaches is a powerful tool to identify new cis-regulatory elements at complex regulated gene loci.

Molecular analysis of the zinc finger transcription factor MAZR

We have recently identified that the BTB domain-containing zinc finger protein MAZR is an important regulator of CD8 expression. MAZR binds to Cd8 enhancers and forced expression of MAZR during T cell development induced variegated CD8 expression, most likely due to co-recruitment of repressor complexes (Bilic et al., 2006, Nature Immunology). This indicates that MAZR regulates chromatin remodeling at the Cd8 gene complex (see Figure 1). We recently generated MAZR-deficient mice to learn more about the role of MAZR in T cells and other cells of the hematopoietic systems. Ongoing studies focus on the analysis of MAZR-deficient mice.

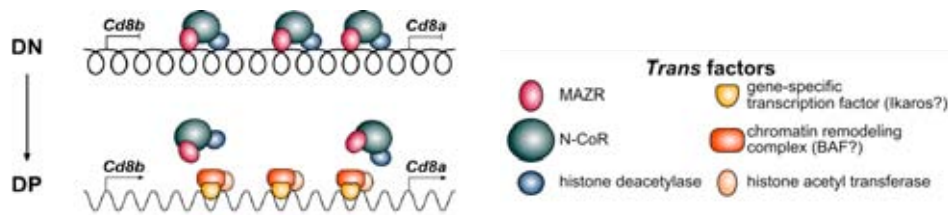


Fig. 1: Model of MAZR function. In DN thymocytes, MAZR binds to the Cd8ab gene complex, and via recruitment of nuclear corepressor (N-CoR)-containing complexes represses CD8 expression. In DP thymocytes, less MAZR is bound to the Cd8ab gene complex, and thus probably facilitating the recruitment of positive-acting chromatin remodeling complexes (model after Bilic et al., 2006, Nature Immunology, 7, 392).

In addition, to understand the molecular mechanisms of how MAZR regulates the expression of CD8, we are aiming to identify MAZR interacting factor using tandem affinity purification strategies and mass spectroscopy approaches.

The role of histone deacetylases in T cell development and function

Reversible changes in histone acetylation patterns have been shown to accompany many important processes in T cells ranging from VDJ recombination during T cell development to the induction of cytokine expression during Th1/Th2 effector differentiation. Modification of core histones by lysine acetylation is controlled by histone acetyltransferases and histone deacetylases, which are considered as transcriptional co-activators and co-repressors, respectively. Eighteen histone deacetylases (HDACs) have been identified in mammalian organisms, however dissecting individual roles for each member of the HDAC family in specific cell lineages and tissues remains a major scientific challenge. In a close collaboration with the research group of Christian Seiser (Max F. Perutz Laboratories, Vienna) we are analyzing the role of certain members of the HDAC family in T cells.

Transcriptional control of memory-phenotype T cell development by PLZF

Peripheral CD4⁺ and CD8⁺ T cell subsets have been traditionally divided into naïve CD44^{lo}CD62L⁺ and memory CD44^{hi}CD62L⁻ populations, while the surface expression phenotype of the latter population also resembles recently activated T cells. However, the memory T cell subset is not a population consisting only of true antigen-specific memory cells that developed in response to a foreign antigen. Rather, the memory population contains in addition a variety of different T lymphocyte subsets, some of which acquired their memory phenotype through homeostatic proliferation, while others have immediate effector function and may play a role in the front-line defense against certain bacterial infections (“innate-like” T cells). The signaling pathways and transcriptional networks that regulate the developmental cell fate decisions between conventional and innate-like memory-phenotype T cell subsets are not well understood.

We recently observed that the BTB domain-containing transcription factor PLZF (promyelocytic leukemia zinc finger) is expressed in CD44^{hi} memory-phenotype but not in naïve CD44^{lo} CD4⁺ T cells. To investigate a potential role of PLZF in either the generation and/or function of the CD44^{hi}CD4⁺ T cell subset, we performed gain-of-function experiments and generated PLZF transgenic mice. With this experimental approach we could show that PLZF is a crucial transcriptional regulator that induces the development of CD44^{hi} memory-phenotype T cells with innate-like characteristics (Ref. 9). Ongoing studies address mechanistic aspects of how PLZF regulates the development of CD44^{hi} memory-phenotype T cells.

TEC FAMILY KINASES AND SIGNALING PATHWAYS IN IMMUNE CELLS

Members of the Tec kinase family (Bmx, Btk, Itk, Rlk and Tec) constitute the second largest family of non-receptor tyrosine kinases and are preferentially expressed in the hematopoietic system (see Figure 2). A large number of studies have shown important roles for these kinases in the lymphoid system. Furthermore, mice with combinatorial deletions of Tec family kinases revealed both unique and redundant functions in B cells (Tec, Btk) and T cells (Rlk, Itk). In ongoing studies we are investigating in more detail the role of Tec family kinases in T cells.

Although Tec family kinase members are also expressed in myeloid cells, little is known about their function in this lineage. To learn more about the role of Tec kinases in myeloid cells, we are analyzing the function of primary myeloid cell lineages such as mast cells and monocytes/macrophages lacking single and/or multiple Tec kinase family members. Our studies will contribute to a better understanding of the immunomodulatory role of Tec kinases in cells of the immune system, and may on the long-term also help to indicate strategies for potential therapeutic intervention.

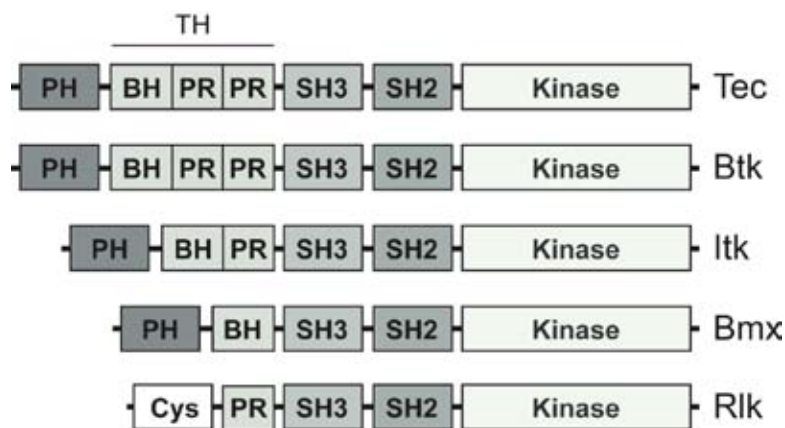


Fig. 2: Modular Structure of Tec Family Kinases. The pleckstrin homology (PH) domain located at the N-terminus of the molecule is the most characteristic feature of Tec family kinases (with the exception of Rlk). PH domains are able to bind phospholipids (or proteins) and are thereby involved in the recruitment of Tec kinases to the membrane. The Tec homology (TH) domain is formed by a Btk homology (BH) motif and by one or two proline-rich (PR) regions, and has been implicated in the auto-regulation of Tec kinases. The TH domain is followed by Src homology (SH) domains SH3 and SH2. The N-terminal palmitoylated cysteine-rich sequence of Rlk mediates membrane anchoring (drawing is taken from Ref. 12).

The role of Tec family kinases in T cells

Itk and the GDP/GTP guanine exchange factor Vav1 act in similar T cell activation pathways. Both molecules interact with members of the Cbl family of E3 ubiquitin ligases, and signaling defects in Vav1^{-/-} T cells are rescued upon deletion of Cbl-b. We investigated the relation between Itk and Cbl-b or Vav1 by generating Itk/Cbl-b and Itk/Vav1 double-deficient mice. We could show that Itk and Vav1 act mechanistically similar in peripheral T cells, since the defects in Itk^{-/-} T cells, like in Vav1^{-/-} T cells, are rescued if cells are released from the negative regulation mediated by Cbl-b. Moreover, our studies revealed that the combined activity of Itk and Vav1 is required for proper T cell development and the generation of the peripheral T cell pool (Ref. 8).

Moreover, in collaboration with Edvard Smith (Karolinska Institute, Sweden) we recently characterized the transcriptome of Itk-deficient T-cells, including CD4⁺ and CD8⁺ subsets, using Affymetrix microarrays, and provided a general overview about the global transcriptional changes in the absence of Itk (Ref. 11). Currently, differentially expressed genes are analyzed.

The role of Tec family kinases in myeloid cells

We identified important processes in myeloid cells that are regulated by Tec kinases. We demonstrated that Tec is a crucial regulator of mast cell function, since Tec-deficient mast cells have an impaired effector function upon FcεRI stimulation (Ref. 13). In addition, we showed that Tec and Btk regulate M-CSFR signaling-induced macrophage survival (Ref. 7). Currently, we investigate in detail how macrophage function is regulated by Tec family kinases.

Grants

START-Program, Ministry of Education, Science and Culture (BMBWK) „Molecular mechanisms of Lymphocyte Development and Activation“ 11/2001 – 10/2008

FWF (Austrian Science Fund) SFB F-2301 „Mechanisms of Establishment and Maintenance of Immunological Tolerance“ SFB Administration and Coordination Project 3/2005 – 8/2009

FWF (Austrian Science Fund) SFB F2305, „Signaling requirements for the induction and maintenance of T cell tolerance“ 3/2005 – 8/2009

EU Marie Curie Research and Training Network on “Chromatin Plasticity” (MRTN-CT-2006-035733) 10/2006 – 10/2010

FWF (Austrian Science Fund) P-19930-B10 “Molecular characterization of the transcriptional regulator MAZR” 8/2007 – 7/2010

FWF (Austrian Science Fund) & MUW DK-W12 „Inflammation and Immunity“, subproject 2, since 10/2007
WWTF (Vienna Science, Research and Technology Fund) (LS09-031) „Epigenetic regulation of T cell development and function“, since 10/2009

Theses**Diploma theses**

Matthias Hombauer: Molecular studies on the transcriptional regulation of the Cd8ab gene loci during T cell development (completed November 2008)

Beatrice Grabner: The regulation of T cell effector differentiation by PLZF (since September 2008)

PhD Theses

Julia Raberger: Studies on the role of the tyrosine kinase Itk in T cell development and function (completed March 2009)

Shinya Sakaguchi: Loss of function analysis of the BTB zinc finger protein MAZR (completed March 2009)

Hammad Hassan: Molecular mechanism of Cd8 enhancer function (since July 2006)
 Wolf Henning Gebhardt: Identification of MAZR binding factors (since April 2007)
 Derya Köprülü : The role of Tec family kinases in macrophages (since September 2007)
 Anastasia Abramova: The role of MAZR in myeloid cells (since October 2007)
 Roland Tschismarov: Regulation of T cell effector function (since November 2008)
 Matthias Hombauer: The role of MAZR in peripheral T cells (since January 2009)

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