Rag defects and thymic stroma: lessons from animal models

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Thymocytes and thymic epithelial cells (TECs) cross-talk is essential to support T cell development and preserve thymic architecture and maturation of TECs and Foxp3+ natural regulatory T cells. Accordingly, disruption of thymic lymphostromal cross-talk may have major implications on the thymic mechanisms that govern T cell tolerance. Several genetic defects have been described in humans that affect early stages of T cell development (leading to severe combined immune deficiency (SCID) or late stages in thymocyte maturation (resulting in combined immunodeficiency). Hypomorphic mutations in SCID-causing genes may allow for generation of a limited pool of T lymphocytes with a restricted repertoire. These conditions are often associated with infiltration of peripheral tissues by activated T cells and immune dysregulation, as best exemplified by Omenn syndrome (OS). In this review, we will discuss our recent findings on abnormalities of thymic microenvironment in OS with a special focus of defective maturation of TECs, altered distribution of thymic dendritic cells and impairment of deletional and non-deletional mechanisms of central tolerance. Here, taking advantage of mouse models of OS and atypical SCID, we will discuss how modifications in stromal compartment impact and shape lymphocyte differentiation, and vice versa how inefficient T cell signaling results in defective stromal maturation. These findings are instrumental to understand the extent to which novel therapeutic strategies should act on thymic stroma to achieve full immune reconstitution.

Keywords: thymus, Rag deficiency, Omenn and leaky SCID models, central tolerance, thymic reconstitution, thymic cross-talk

INTRODUCTION

Thymocytes and thymic epithelial cells (TECs) cross-talk is essential to support T cell development and preserve thymic architecture and maturation of TECs and Foxp3+ natural regulatory T (nTreg) cells. In particular, deletion of self-reactive thymocytes in the thymic medulla is based on the recognition of self-antigens that are presented by medullary TECs (mTECs) and thymic dendritic cells (DCs). In this process, a key role is played by the autoimmune regulator (AIRE), a transcription factor expressed by a subset of mature mTEC that drives the expression of tissue-restricted antigens (TRAs), thus mediating negative selection of autoreactive mature mTEC that drives the expression of tissue-restricted anti-regulator (AIRE), a transcription factor expressed by a subset of cells (DCs). In particular, deletion of self-reactive thymocytes in the thymic medulla is based on the recognition of self-antigens that are presented by medullary TECs (mTECs) and thymic dendritic cells (DCs). In this process, a key role is played by the autoimmune regulator (AIRE), a transcription factor expressed by a subset of mature mTEC that drives the expression of tissue-restricted antigens (TRAs), thus mediating negative selection of autoreactive thymocytes (1, 2). In addition, mature TECs from the Hassall corpuscles secrete thymic stromal lymphopoietin (TSLP), a cytokine that acts through thymic DCs and activates them to instruct self-reactive T cells to be diverted into Foxp3+ nTreg cells (3). These findings highlight the critical role played by the thymus not only in the generation of a diversified and functional T cell repertoire, but also in the prevention of autoimmune manifestations. To this end, defects in T cell development represent a valuable model for studying mechanisms by which severe impairment in thymopoiesis may impinge on thymic stromal cell homeostasis and deletional and non-deletional mechanisms (4). In particular, severe combined immune deficiency (SCID) includes a heterogeneous group of genetic disorders that abolish T cell development at early stage of T cell differentiation by affecting survival of lymphoid progenitors (as in adenosine deaminase deficiency and reticular dysgenesis), interleukin (IL)-mediated expansion of lymphoid progenitors (as in patients with mutations in the γ common chain, JAK3, or IL-7 receptor), V(D)J recombination [as in recombination activating gene (Rag)1 and 2, and Artemis deficiency] in lymphoid precursors and signaling through the pre-T cell receptor (mutations of CD3ε, CD3ζ, CD3γ, and CD45) (5). Null mutations in these SCID-causing genes are associated with a virtual lack of circulating T lymphocytes. However, hypomorphic mutations in the same genes may allow for development of a restricted number of T lymphocytes with limited repertoire diversity, which, when exported to the periphery, may infiltrate target tissues and cause autoimmunity and organ dysfunction, as in patients with Omenn syndrome (OS) (6–10). Finally, defects in T cell development that compromise thymocyte development beyond the CD4+ CD8+ [double-positive (DP)] stage result in combined immunodeficiency with residual number of circulating T lymphocytes that show abnormal phenotype and function. In particular, impaired production of single-positive (SP) CD4+ cells is observed in major histocompatibility complex (MHC) class II deficiency, whereas generation of SP CD8+ lymphocytes is compromised in ZAP70
deficiency (11, 12). Association of these conditions with immune dysregulation has been reported, although not as frequently as in OS due to hypomorphic mutations in SCID-causing genes (13).

In this review, we will focus the discussion on the contribution of thymic microenvironment on the pathogenesis of peripheral immune pathology in the presence of residual V(D)J recombination activity. To this end, we will discuss findings observed in Rag2/R229Q/R229Q and Rag1/S723C/S723C mutant mice, which represent a valuable model of OS and atypical SCID, respectively (14–18). Collectively, we provide evidence that abnormalities of thymic stroma secondary to impaired development of T lymphocytes may affect key mechanisms of immune tolerance and ultimately result in severe manifestations of immune dysregulation.

MOUSE MODELS OF LEAKY SCID AND OS

Mutations of Rag genes result in a variety of clinical and immunological phenotypes. In particular, while null mutations cause a severe block in T and B cell development (T− B− SCID), hypomorphic Rag1 and Rag2 mutations may cause a spectrum of phenotypes, including OS, atypical SCID, combined immune deficiency with expansion of oligoclonal, activated T cells, profound B cell lymphopenia, and yet significant increase of CD11c+ plasmacytoid DCs (pDCs), and a decreased proportion of CD11c+CD45RA− conventional DCs (cDCs) was demonstrated in Rag2R229Q/R229Q mice (17). A severe reduction of both cDCs and pDCs was demonstrated in Rag2R229Q/R229Q mice, and was associated with a random distribution of these DC subsets throughout the thymus. Furthermore, a significant reduction in the expression of MHC-II and CD86 was found in both DC subset populations, suggesting impairment in DC maturation process (28). Of note, impaired maturation of mTECs, defective expression of AIRE and reduced number of thymic DCs have been shown in Rag2R229Q/R229Q mice, as well as in patients with hypomorphic Rag mutations (17, 27). These defects inevitably lead to a severe impairment in the process of negative selection of autoreactive T cells.

Unexpectedly, abnormalities of thymic DCs, which are involved in promoting negative selection of self-reactive thymocytes and in the generation of nTregs, were also demonstrated in both mutant models. In particular, a relative abundance of CD11cint CD45RA+ plasmacytoid DCs (pDCs), and a decreased proportion of CD11c+ CD45RA− conventional DCs (cDCs) was demonstrated in Rag1S723C/S723C mice (17). A severe reduction of both cDCs and pDCs was demonstrated in Rag2R229Q/R229Q mice, and was associated with a random distribution of these DC subsets throughout the thymus. Furthermore, a significant reduction in the expression of MHC-II and CD86 was found in both DC subset populations, suggesting impairment in DC maturation process (28). Of note, impaired maturation of mTECs, defective expression of AIRE and reduced number of thymic DCs have been also reported in patients with hypomorphic mutations of genes involved in early stages of T cell development (29, 30). While the mechanisms accounting for thymic DC abnormalities in mice and patients with hypomorphic Rag mutations remain poorly defined, they have important consequences on maintenance of immune homeostasis. In particular, cDCs have been described to contribute to the generation of nTregs (31). Consistent with this, a reduced number of Foxp3+ nTreg cells have been observed in both Rag1S723C/S723C and Rag2R229Q/R229Q mice, as well as in patients with Rag-dependent OS (16, 17, 30).

Altogether, the study of animal models carrying hypomorphic Rag mutations has demonstrated that defective T cell lymphopoiesis affects maturation and function of thymic stroma, and impinges on both deletional and non-deletional mechanisms of immune tolerance, thereby providing important insights on the pathophysiology of OS.

ANIMAL STUDIES TO TARGET THYMIC STROMA IN Rag DEFICIENCIES

GENE THERAPY IN Rag1 KNOCK-OUT MICE

An additional demonstration of the importance of thymic lymphostromal cross-talk has been provided by recent data
In this particular model, the majority of Rag1 therapy in Rag1 demonstrating that inefficient T cell reconstitution following gene expression leads to poor reconstitution of T and B cells and is insufficient to restore thymic stroma architecture, maturation of AIRE and TSA-expressing mTECs, and induction of both T and B cell tolerance. In this scenario, development of a limited number of T and B lymphocytes and inability to maintain efficient tolerance checkpoints lead to the development of OS-like manifestations. Overall, these data illustrate the importance of T cell reconstitution for restoring the differentiation and maturation of TECs, and emphasize the relevance of thymic stroma in ensuring immune tolerance and preventing thymic egress of autoreactive T cell clones.

**ANTI-CD3ε mAb TREATMENT IN Rag2<sup>R229Q/R229Q</sup> MOUSE MODEL OF OS**

As previously described, OS is an atypical SCID in which the coexistence of immunodeficiency and autoimmunity remains an intriguing aspect that needs to be further investigated. Thanks to availability of the Rag2<sup>R229Q/R229Q</sup> mouse model, we have studied various mechanisms that contribute to the pathogenesis of autoimmune manifestations of OS. We have demonstrated that in addition to hypomorphic Rag defect leading to generation of a limited number of T cells, severe defects in thymic epithelial compartment occur, which contribute to the escape of autoreactive T cells that invade the periphery triggering autoimmunity (24). This model represents also a valuable tool to evaluate the effects of TCR signaling on maturation of the thymic stromal compartment. To this end, we evaluated the in vivo effect of anti-CD3ε monoclonal antibody (mAb) administration in neonatal and adult mice. While no significant changes were noticed in the thymus of adult treated mice, injection of anti-CD3ε mAb at neonatal age resulted in a dramatic amelioration of the epithelial compartment and peripheral immunopathology. In particular, treatment was associated with a marked reduction in the frequency of effector/memory T cells in the periphery and a significant decrease in interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) production by peripheral T cells. These changes were paralleled by significant modification in thymus morphology, with appearance of distinct areas of CMD and significant improvement of the medullary/cortical ratio (27). Double staining for CK5 and CK8 further confirmed these findings by revealing the presence of well-defined cortical and medullary areas showing that anti-CD3ε mAb treatment enforces maturation of TECs leading to compartmentalization of CK8<sup>+</sup>CK5<sup>−</sup> cTECs and CK8<sup>−</sup>CK5<sup>+</sup> mTECs (Figure 2A). Moreover, we have described an increase in the presence of UEA-1<sup>+</sup> mature mTECs clusters was not fully restored.
Furthermore, treatment with anti-CD3ε mAb normalized the frequency while did not change the absolute number of total thymic DCs and significantly increased MHC-II expression in this population normally down-regulated in Rag2<sup>R229Q/R229Q</sup> mice respect to WT counterpart (Figure 2B). More interestingly, anti-CD3ε mAb treatment induced a redistribution of the two thymic DCs main subsets CD8<sup>−</sup> (myeloid) and CD8<sup>+</sup> (lymphoid) (Figure 2C). The improvement of thymic stroma architecture and maturation were associated with a reduction in tissue infiltrates, as demonstrated by the reduced frequency of CD4<sup>+</sup> and CD8<sup>+</sup> cells in the skin, gut, lung, and liver. Altogether, these data indicate that treatment with anti-CD3ε mAb has a beneficial effect
on thymic stroma and on peripheral immunopathology, and may pave the way for similar therapeutic modalities aiming at improving immune function and reducing signs of immune dysregulation in patients with SCID characterized by poor thymic maturation, while waiting for definitive treatment based on hematopoietic cell transplantation.

**CONCLUSION**

Thymocytes and TEC crosstalk are fundamental for the maintenance of thymic architecture and function. Investigation on thymic morphology and immunophenotype in SCID patients and in parallel analysis of murine models of OS and Leaky SCID have revealed the extent to which altered thymic crosstalk might lead to immune dysregulation and ultimately cause peripheral immunopathology. Thymic stromal improvement and amelioration of peripheral immunopathology upon anti-CD3ε mAb administration in OS mouse model have further highlighted the contribution of thymic stroma in the pathogenesis of immune dysregulation. In parallel, poor immunological reconstitution observed in the preclinical study of gene therapy caused by inadequate Rag1 expression has further emphasized the relevance of thymic stromal thymic compartment in the induction and maintenance of immune tolerance. Overall these findings further define the role of thymic epithelium in immune reconstitution and indicate that cTECs and mTECs full restoration has to be achieved to prevent immune dysregulation.

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