Rag defects and thymic stroma: lessons from animal models

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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 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

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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 Rag2R229Q/R229Q and Rag1S723C/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 immunodeficiency with expansion of TCRαβ+ T cells, and combined immunodeficiency with granuloma and/or autoimmunity (CID-G/A) despite their common molecular underlying the disease (19–25). While all of these conditions associated with hypomorphic Rag mutations are characterized by residual development of T (and in some cases, B) lymphocytes, some of them (especially OS and CID-G/A) present with prominent immune dysregulation. However, the cellular and molecular mechanisms underlying autoimmunity have remained poorly defined until recently, when animal models of OS and leaky SCID have become available (16, 17, 26). In particular, Khiong and colleagues have reported on a spontaneously occurring mouse mutant (named MM) in which a homozygous point mutation in the Rag1 gene (R972Q) was associated with a high proportion of memory T cells in the periphery. Although MM mice showed skin redness when shaved, no T cells infiltration was observed in the tissues and no obvious signs were reported, making this mutant strain a valuable model of OS and atypical SCID, respectively (14–18).

In particular, both Rag1S723C/S723C and Rag2R229Q/R229Q mice displayed altered maturation of mTECs, as indicated by the virtual absence of claudin-4 (Cl4d) and Ulex europaeus Agglutinin 1 (UEA-1) ligand. Furthermore, analysis of cytokine (ck) expression in the thymus revealed abundance of CKR− CK5+ cells, which represent immature TEC progenitors and a severe reduction of CK8− CK5+ mTECs. FACS analysis labeling CD45− EpCAM+ thymic stromal cells with UEA-1 and Ly51 specific antibodies for mTECs and cTECs, respectively, have demonstrated the increased frequency of cTECs with consequent reduction in mTEC compartment in Rag2R229Q/R229Q mouse compared to WT (Figure 1A). However, all epithelial populations were significantly diminished in number given the dramatic reduction in total thymic cellularity (Figure 1B). Defective maturation of mTECs in Rag1S723C/S723C and Rag2R229Q/R229Q mice was associated with severe reduction of AIRE-expressing cells and markedly reduced expression of TRAs, such as cytochrome P450, insulin 2, glutamic acid decarboxylase 67, and fatty acid-binding proteins (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 lymphopoiensis 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 demonstrating that inefficient T cell reconstitution following gene expression of a well-defined CMD and a normal distribution of cTEC, rescue of thymopoiesis and thymic stroma architecture, with presence of wild-type bone marrow cells into Rag1 of mature mTECs expressing AIRE. By contrast, transplantation of human Rag1 cDNA driven by ubiquitous and cell type-restricted promoters showed low level of T cell reconstitution. In this setting, treatment was associated with a marked reduction in the number of 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 effect of anti-CD3ε mAb treatment in Rag2R229Q/R229Q 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 Rag2R229Q/R229Q 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).

Anti-CD3ε mAb treatment in Rag2R229Q/R229Q 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 Rag2R229Q/R229Q 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+CK5− cTECs and CK8−CK5+ mTECs (Figure 2A). Moreover, we have described an increase in the presence of UEA-1+ mature mTECs clusters was not fully restored.
FIGURE 2 | Anti-CD3ε mAb administration enhances thymic epithelium compartmentalization and maturation and modifies thymic DCs frequency and distribution in Rag2<sup>R229Q/R229Q</sup> newborns. (A) Left panel shows representative immunohistochemistry from WT thymus displaying a well-defined corticomedullary differentiation and normal compartmentalization of CK8<sup>+</sup>CK5<sup>−</sup> cTECs (CK8, blue) and CK8<sup>−</sup>CK5<sup>+</sup> mTECs (CK5, brown). mTECs show mature morphology with large cytoplasm and delicate CK5 positivity with rare double-positive CK8<sup>+</sup>CK5<sup>+</sup> immature TECs disposed along the corticomedullary junction (asterisk within inset). Corticomedullary differentiation and maturation of TECs are profoundly impaired in Rag2<sup>R229Q/R229Q</sup> mouse (middle panel) in which immature TECs expressing both CK5 and CK8 are highly represented (asterisk within inset). Anti-CD3ε mAb administration enforces maturation of TECs leading to compartmentalization of CK8<sup>+</sup>CK5<sup>−</sup> cTECs and CK8<sup>−</sup>CK5<sup>+</sup> mTECs (right panel), although mTECs are still closely packed and irregularly distributed with intense CK5 positivity as compared to the normal medulla (detail of morphology within inset). Double immunohistochemical staining: CK5 (brown staining) and CK8 (blue staining). (m, medulla; c, cortex; scale bars corresponds to 200 and 50 µm for 10× and 40× (insets) original magnification, respectively). (B) Graphic representation of the percentage and absolute number of CD11c<sup>+</sup> cells in the thymus of all mice analyzed (WT, n = 7; Rag2<sup>R229Q/R229Q</sup>, n = 7; Rag2<sup>R229Q/R229Q</sup> + anti-CD3 n = 9). The last graph on the right indicates mean fluorescence intensity (MFI) of MHC-II expression on total CD11c<sup>+</sup> cells in all mice analyzed (WT, n = 5; Rag2<sup>R229Q/R229Q</sup>, n = 6; Rag2<sup>R229Q/R229Q</sup> + anti-CD3 n = 4). (C) Representative dot plot indicating the distribution of myeloid (CD8<sup>−</sup>) and lymphoid (CD8<sup>+</sup>) populations in the gate of CD11c<sup>+</sup> cells (upper panel). Statistics of the percentage and the absolute numbers of CD8<sup>+</sup> and CD8<sup>−</sup>CD11c<sup>+</sup> in all mice analyzed (WT, n = 5; Rag2<sup>R229Q/R229Q</sup>, n = 4; Rag2<sup>R229Q/R229Q</sup> + anti-CD3 n = 4) (lower panel). Groups were analyzed with Prism software (GraphPad) using a two-tailed Mann-Whitney unpaired test. Data are presented as mean ± SD. P-values of < 0.05 were considered significant.

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.
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