FoxP3: A genetic link between immunodeficiency and autoimmune diseases

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Abstract

It has long been observed that patients with autoimmune diseases also have immune deficiency. How these two opposite extremes of immunity can be found in the same individual is largely unclear. Here we review the evidence that a FoxP3 defect may provide a critical link between autoimmunity and immune deficiency. Disruption of FoxP3 results in severe autoimmune syndromes in both human and mice. Bone marrow chimera experiments indicate that FoxP3 defects in both hematopoietic and non-hematopoietic cells are required for the development of severe autoimmune disease. FoxP3 mutation in the hematopoietic cells impairs the development of regulatory T cells (Treg). Our data demonstrate that the mutation in non-hematopoietic cells results in deficient thymopoiesis. Defective T cell production may be an underlying cause of T cell hyperproliferation, which together with Treg defects, may lead to fatal autoimmune disease in mouse and man.

Keywords: Homeostatic proliferation; Thymopoiesis; IPEX; Scurfy

1. Introduction

Autoimmunity is defined as over-reactive immune responses against self tissues. Paradoxically, in a number of autoimmune diseases, immune deficiency coexists with autoimmunity [1–5]. Recent studies on lymphopenia-induced homeostatic proliferation, which results in a generation of memory-like cells [6–8], suggested a mechanism to reconcile this paradox. As demonstrated in NOD mice, a mouse model of human type 1 diabetes [9,10], lymphopenia was associated with increased proliferation of T cells, which may be causatively related to the development of type 1 diabetes.

Lymphopenia can be induced by either environmental factors such as chemotherapy [11] and irradiation [6–8] or theoretically, by defective production or survival of T cells. Our recent studies in the Scurfy mouse model demonstrate that the FoxP3 gene may provide a genetic link between immune deficiency and autoimmune diseases.

2. FoxP3 mutation and autoimmune diseases

The Scurfy mutation occurred spontaneously at the Oak Ridge National Laboratory in the late 1940s. Only the male was found to be affected and the mutant male
mice usually died within one month after birth due to the severe autoimmune disease characterized by lymphadenopathy, splenomegaly, and massive lymphocyte infiltrations in various organs [12]. Early studies on Scurfy mice showed that mutant CD4⁺CD8⁻ T cells were sufficient to transfer the disease to a syngeneic immune deficient host, indicating that CD4 helper T cells are the primary cause of the deadly autoimmune disease in the Scurfy mice [13]. Subsequent molecular genetic studies revealed a frame shift mutation in the 8th exon of the FoxP3 gene in the Scurfy mice as the underlying genetic cause of the disease [14]. Analogous mutation of human FoxP3 was found to be responsible for Immunodysregulation, polyendopathy, enteropathy, X-linked diseases (IPEX) [15–18]. In both mouse and human, the FoxP3 mutation is responsible for the most aggressive autoimmune diseases that resulted in early lethality.

The most clearly elucidated function of FoxP3 is T cell-intrinsic. FoxP3 is found to be predominantly expressed in the CD4⁺CD25⁺ regulatory T cells (Treg), and ectopic expression of FoxP3 in CD4⁺CD25⁻ T cells is sufficient to convert them into Treg with strong suppressor activity [19–21]. More importantly, targeted mutation of FoxP3 in hematopoietic cells is both necessary and sufficient to ablate Treg development [21]. Thus, FoxP3 is now regarded as a master regulator for the lineage differentiation and function of Treg.

However, several lines of evidence also suggest that defective Treg development alone may be insufficient to initiate such severe autoimmune diseases as those observed in Scurfy mice and IPEX patients. First, transgenic expression of wild type FoxP3 under the Ick promoter did not rescue the autoimmunity in Scurfy mice, although it is unclear whether modest elevation of the FoxP3 gene in the spleen of one founder line can fully restore Treg function [22]. Second, irradiation chimeras using bone marrow from Scurfy mice to reconstitute irradiated SCID mice did not lead to the development of autoimmune disease [23]. Because irradiation leads to the generation of T cells from SCID mice, we have reproduced the results using RAG-1 and RAG-2-deficient hosts. In both cases, we showed that complete reconstitution of T cells from the Scurfy bone marrow did not lead to Scurfy-like disease. The B6.RAG-1/Scurfy chimera mice lived for more than one year after reconstitution. Although the RAG-2/Scurfy mice lived less than 20 weeks, the pathogenesis was distinct from Scurfy mice by the lack of characteristic lymphoproliferation. These results demonstrate a T cell-extrinsic function of FoxP3.

3. Defective T cell production caused by T cell-extrinsic mutation of FoxP3

In our analysis of the immunological basis of autoimmune diseases associated with FoxP3 mutation, we observed a very substantial reduction in thymic cellularity. The reduction was caused by a reduction in proliferation of immature thymocytes that lack both CD4 and CD8 co-receptors. In order to determine whether the defective thymopoiesis was associated with T cell activation in the Scurfy mice, we crossed the FoxP3 mutant allele into the RAG-2-deficient background. Even though T cell development was arrested at an early (DN3, CD25⁺CD4⁻) stage, RAG-2-deficient, FoxP3 mutant mice showed defects in early thymocyte proliferation. These results demonstrate that defective thymopoiesis is not a secondary consequence of T cell activation in the Scurfy mice. Interestingly, in bone marrow chimera mice consisting of either FoxP3 WT or FoxP3 mutant bone marrow cells, thymopoiesis was normal. Thus, defective thymopoiesis is not due to a T cell-intrinsic defect of FoxP3. Conversely, a mutation of FoxP3 in the thymic stroma cells was necessary and sufficient to cause defective thymopoiesis.

We have obtained several additional lines of evidence to support a critical role for FoxP3 in thymopoiesis. First, we showed that FoxP3 was expressed at high levels in cortical thymic epithelial cells. Thus, on a per cell basis, FACS-sorted thymic epithelial cells had higher levels of FoxP3 mRNA than the total thymocytes. Immunofluorescence analysis revealed expression of FoxP3 in the cortical but not medulla thymocytes. Second, FoxP3 repressed expression of ErbB2, which was implicated in inhibiting thymocyte development [24]. We observed that the transfection of the thymic epithelial cell line repressed ErbB2 expression, at least in part by repressing its promoter activity. Conversely, we showed that ErbB2 expression was significantly elevated in the thymus of the Scurfy mice. Thirdly, we showed that Herceptin, which cross-reacts with mouse ErbB2, can partially restore proliferation of DN thymocytes.

Taken together, our data [25] demonstrate that FoxP3 mutation has a direct effect on the production of thymocytes in addition to its known function in the generation of Treg. Our data thus provide a genetic link between immunodeficiency and autoimmune diseases (Fig. 1).

It is worth noting that an elegant study by Fontenot and colleagues [26] has demonstrated that targeted mutation of FoxP3 by CD4 promoter-driven Cre appears necessary and sufficient to cause fatal autoimmune diseases. While these data raised a serious issue as to whether the FoxP3 defect in non-T lineage is required
Take-home messages

- The autoimmune diseases associated with FoxP3 mutation require both T cell-intrinsic defects that result in defective development of regulatory T cells and T cell-extrinsic defects in thymocyte production.
- Defective T cell production is a general feature of multiple autoimmune diseases. FoxP3 is likely the first genetic link between autoimmune diseases and immunodeficiency.
- Homeostatic proliferation may be the underlying immunological mechanism linking immune deficiency to autoimmune diseases.

References

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