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Autoimmunity Reviews xx (2005) xxx–xxx
AUTOIMMUNITY REVIEWS
www.elsevier.com/locate/autrev

FoxP3: A genetic link between immunodeficiency and autoimmune diseases

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Received 15 September 2005; accepted 6 October 2005

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 I diabetes [9,10], lymphopenia was associated with increased proliferation of T cells, which may be causatively related to the development of type I 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

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1568-9972/$ - see front matter © 2005 Published by Elsevier B.V.
doi:10.1016/j.autrev.2005.10.008

mice usually died within one month after birth due to the severe autoimmune disease characterized by lymphadenpathy, 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 lck 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, $CD25^+CD44^-$) 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

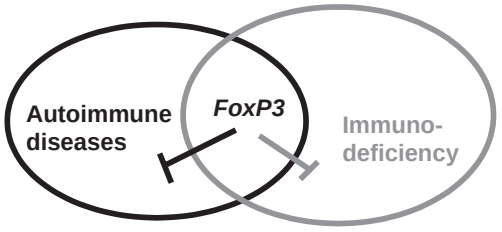

Fig. 1. *FoxP3* as a genetic link between autoimmune diseases and immunodeficiency.

for pathogenesis, it should be pointed out that lineage specific expression of Cre in this transgenic line has not been fully established. In fact, we have demonstrated that the CD4 gene is actively transcribed in the thymic epithelial cells [25]. Further work is needed to reconcile our data with those published by the Fontenot group.

The defects in thymic T cell production are also observed in some other autoimmune syndromes. Thus, in the human, DiGeorge syndrome can lead to autoimmune diseases [1–5]; thymoma is commonly associated with myasthenia gravis [27,28], while thymic hypoplasia is associated with autoimmune hemolytic anemia and juvenile pemphigoid [29]. Defective thymopoiesis and the export of mature T cells have also been reported in both RA [30] and MS [31] patients. The diabetes-prone BB rats have severe defects in thymocyte development due to mutations of the IAN gene family members [32,33].

How does the defective thymopoiesis in the Scurfy mice contribute to the pathogenesis of autoimmunity? Theoretically, a reduced T cell production may cause lymphopenia-driven proliferation of T cells in the periphery. In support of this notion, we have obtained preliminary data which showed strong homeostatic proliferation of T cells in the periphery of mice with defective *FoxP3* in non-hematopoietic cells (our unpublished observation). The dysregulated homeostatic proliferation was observed in the CD4 cells from RA patients [34]. Likewise, it is well established that lupus patients have severe lymphopenia [35]. Y-chromosome-associated murine lupus was reported to be associated with lymphopenia and homeostatic proliferation of T cells [36]. Thus, understanding the molecular checkpoints of homeostatic proliferation may have a general significance for autoimmune diseases.

Acknowledgements

We thank Lynde Shaw for editorial assistance. This work is supported by grants from the National Institutes of Health, US Department of Defense, and the BRTT award from the State of Ohio.

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.

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