

Regulatory T cells in primary immunodeficiency diseases

Troy R. Torgerson and Hans D. Ochs

Purpose of review

Several primary immune deficiency disorders are associated with autoimmunity and malignancy, suggesting a state of immune dysregulation. Here, we review the role of regulatory T-cell deficits in mediating the immune dysregulation associated with certain primary immune deficiency disorder syndromes.

Recent findings

Systematic studies in primary immune deficiency disorders and their associated animal models have led to an increased understanding of both central and peripheral tolerance mechanisms, and in particular have yielded new insights into regulatory T-cell function, development and maintenance.

Summary

Single-gene defects identified in patients with multiple autoimmune phenomena have defined new primary immune deficiency disorder syndromes in which the primary deficit is in the establishment or maintenance of immune tolerance. The disorder that has been most informative with regard to understanding the function and development of regulatory T cells is forkhead box P3 deficiency, known as immune dysregulation, polyendocrinopathy, enteropathy and X-linked syndrome in humans and *Scurfy* in the mouse. Recent studies in patients with other primary immune deficiency disorders, including autoimmune polyendocrinopathy, candidiasis and ectodermal dystrophy syndrome, CD25 deficiency, STAT5b deficiency, and Wiskott–Aldrich syndrome, have added to our understanding of regulatory T-cell biology. The study of patients with rare primary immune deficiency disorder syndromes provides an unparalleled opportunity to understand mechanisms of autoimmunity and immune tolerance in humans.

Keywords

autoimmunity, forkhead box P3, immune tolerance, primary immune deficiency disorders, regulatory T cell

Abbreviations

APECED	autoimmune polyendocrinopathy, candidiasis and ectodermal dystrophy
FOXP3	forkhead box P3
IPEX	immune dysregulation, polyendocrinopathy, enteropathy and X-linked
PIDD	primary immune deficiency disorder
T_{REG}	regulatory T cell
WAS	Wiskott–Aldrich syndrome
WASP	Wiskott–Aldrich syndrome protein

© 2007 Wolters Kluwer Health | Lippincott Williams & Wilkins
1528-4050

Introduction

Primary immune deficiency disorders (PIDDs) have traditionally been thought of as defects in the cognate or innate immune system that are characterized primarily by recurrent bacterial, viral and fungal infections. Increasingly, it has been recognized that many of these disorders are associated with a higher incidence of autoimmune diseases and malignancies. This dichotomy of overreaction to self-antigens while lacking a robust response against microorganisms suggests that PIDD patients have a ‘dys-regulated’ immune system resulting in a decreased ability to differentiate between self and foreign antigens. The concept of immune dysregulation as a direct cause of autoimmunity in PIDDs has been strengthened by the recent discovery of distinct clinical entities linked to single gene defects resulting in multiple autoimmune phenomena including immune dysregulation, polyendocrinopathy, enteropathy and X-linked (IPEX) syndrome, autoimmune polyendocrinopathy, candidiasis and ectodermal dystrophy (APECED) syndrome, and autoimmune lymphoproliferative syndrome [1–3].

Immune dysregulation, polyendocrinopathy, enteropathy and X-linked syndrome

The year 2007 marks a quarter century since the IPEX syndrome was clinically described [4] and nearly half a century since its spontaneous mouse ortholog, *Scurfy*, was identified [5]. Despite our long-term knowledge of these syndromes, it was their linkage to defects in the forkhead box P3 (*FOXP3*) gene 6 years ago that opened the door for the recent explosion in our understanding of T_{REG} biology. It is now clear that absence of functional FOXP3 protein leads to regulatory T-cell (T_{REG}) deficiency in both humans and mice, and that these syndromes are the phenotypic result of this deficiency.

Clinically, the usual presentation of IPEX is a triad of severe autoimmune enteropathy, early onset endocrinopathies and dermatitis, often leading to death by 2 years

Curr Opin Allergy Clin Immunol 7:515–521.
© 2007 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Division of Pediatric Immunology, Rheumatology and Infectious Diseases,
University of Washington School of Medicine and Children's Hospital and Regional
Medical Center, Seattle, Washington, USA

Correspondence to Hans D. Ochs, MD, Professor of Pediatrics, Jeffrey Modell
Chair of Pediatric Immunology Research, 307 Westlake Avenue North, Suite 300,
Seattle, WA 98109, USA
Tel: +1 206 987 7450; fax: +1 206 987 7310;
e-mail: Hans.Ochs@seattlechildrens.org

Current Opinion in Allergy and Clinical Immunology 2007, 7:515–521

Table 1 Regulatory T cells in primary immunodeficiency diseases

Disorder	Genetic defect	Major clinical features	Regulatory T cell numbers	Regulatory T cell function	Other features
Immune dysregulation, polyendocrinopathy, enteropathy and X-linked syndrome	FOXP3	Triad Enteropathy Endocrinopathy (type I diabetes, thyroiditis) Eczema Other autoimmune disorders (hematologic, renal, etc.)	↓ or Absent	↓↓↓	↑↑↑ IgE ↑ IgA Recurrent infections
CD25 deficiency	CD25/interleukin-2 receptor α	Chronic diarrhea Hepatosplenomegaly Lymphadenopathy Cytomegalovirus pneumonitis	Normal or slightly ↓	Not known	Diabetes ↑ or normal IgE ↓ T cell proliferation
STAT5b deficiency	STAT5b	Dwarfism Prominent forehead Saddle nose High-pitched voice Recurrent herpes virus infections	↓	↓↓↓	PCP pneumonia T cell lymphopenia ↓ natural killer cells and γδ T cells
Autoimmune polyendocrinopathy, candidiasis and ectodermal dystrophy syndrome	AIRE	Hypoparathyroidism Adrenal insufficiency Mucocutaneous candidiasis Gonadal failure	↓	↓↓	Type I diabetes Pernicious anemia Autoimmune hepatitis
Wiskott–Aldrich syndrome	Wiskott–Aldrich syndrome protein	Thrombocytopenia and small platelets Eczema Recurrent infections Autoimmune hemolytic anemia/thrombocytopenia	Normal	↓↓	Malignancies

↓/↑, Decreased/increased relative to healthy controls.

of age unless aggressively managed [6–8]. In the large cohort of IPEX patients that we have characterized (unpublished data), the majority of patients with mutations in FOXP3 also have associated autoimmune disorders including autoimmune hemolytic anemia, renal disease and liver disease, among others (see Table 1). FOXP3-deficient mice, including *Scurfy*, have similarly fulminant systemic autoimmunity, although impressive lymphoproliferative infiltration of liver, spleen and lymph nodes is a prominent feature of the murine disorder which is infrequent in IPEX patients.

FOXP3 is a 431-amino-acid protein that is present primarily in CD4⁺CD25^{high} T cells, although it has recently been shown to be inducibly expressed in any activated human T cell [9,10^{••},11,12]. FOXP3 is a member of the P subfamily of forkhead box transcription factors which have a highly conserved forkhead/winged-helix DNA-binding domain [13]. It was initially shown to exert transcriptional repression on the promoters of key cytokine genes [14,15,16^{••}]; however, recent genome-wide screening approaches suggest that it functions more commonly as a transcriptional enhancer than a transcriptional repressor [17^{••},18^{••}].

Three key structural domains that are critical for the gene repression activity of FOXP3 have been identified based on the location of mutations identified in patients with IPEX. First, the C-terminal forkhead domain, where the

largest number of IPEX missense mutations cluster, is required for DNA binding and nuclear import. Second, the leucine zipper of FOXP3 in the central part of the molecule is required for homo- and possibly heterodimerization of the protein. Third, a repression domain in the N-terminal proline-rich region is required for FOXP3 to suppress transcription [19^{••}]. Recent studies suggest that FOXP3 physically and functionally interacts with other transcription factors including nuclear factor of activated T cells, nuclear factor-κB and AML-1 to modulate gene transcription at key cytokine promoters [15,16^{••},20^{••}].

The initial clue that *Scurfy* mice may lack regulatory cells came from adoptive transfer studies in which CD4⁺ T cells from normal mice were capable of preventing onset of the *Scurfy* phenotype in trans [21]. Subsequently, FOXP3 was linked directly to the generation of naturally arising CD4⁺CD25⁺T_{REG} both in mice [22–24] and in humans [25]. Recent studies in engineered mouse models have demonstrated that, in the absence of *FOXP3* expression, T cells destined to become T_{REG} still acquire the usual phenotypic markers of this lineage including high-level expression of CD25, CTLA-4 and glucocorticoid-induced tumor necrosis factor receptor, but they have no suppressive function [26^{••},27^{••}]. Instead, they develop a gene expression profile similar to effector/cytotoxic T cells [26^{••}]. Humans with IPEX typically have normal numbers of CD4⁺CD25⁺ T cells,

but it is not yet clear whether these cells demonstrate a similar effector/cytotoxic profile.

Treatment of IPEX has focused on suppression of unregulated effector T cells using cyclosporine A or tacrolimus (FK506), with or without steroids [6,7,28]. Sirolimus (rapamycin) has recently been found to be effective in treating IPEX, possibly due to its ability to preferentially inhibit growth of effector T cells relative to growth of T_{REG} [29,30,31[•]]. Stem cell transplantation is capable of restoring a stable population of T_{REG} cells and is currently the only effective cure for IPEX patients [32,33,34[•]]. Both complete and reduced intensity conditioning protocols have been reported as successful, although reduced intensity regimens seem to be associated with better survival.

CD25 deficiency

For some time it has been known that mice lacking the α chain of the interleukin-2 receptor (CD25) have a dramatic phenotype with splenomegaly, lymphadenopathy, severe colitis and autoimmune hemolytic anemia that is reminiscent of the *Scurfy* mouse [35]. It was therefore not unexpected to find that humans who lack CD25 have an IPEX-like phenotype as well.

To date, two unrelated patients with CD25 deficiency (Online Mendelian Inheritance in Man #606367) have been described in the literature [36,37^{••},38]. In each case, inheritance was found to be homozygous recessive leading to a complete lack of CD25 protein expression on activated T cells. One patient, the product of consanguineous parents, was homozygous for a 4-bp pair deletion in the coding region of *CD25* that causes a frameshift and early termination codon within the CD25 protein [36,38]. The other patient had compound heterozygous mutations in the *CD25* gene that led to a frameshift on one allele and a premature stop codon on the other [37^{••}].

Similar to the clinical manifestations of IPEX, both affected patients developed severe, chronic diarrhea and villous atrophy in infancy (one at 6 weeks and the other at 8 months of age) [36,37^{••}]. One also developed early onset insulin-dependent diabetes and later developed eczema as well [37^{••}]. Subsequently, both patients developed autoantibodies, hepatosplenomegaly, lymphadenopathy and lymphocytic infiltrates in various organs (gut, liver, etc.) indicative of ongoing immune dysregulation (see Table 1) [36,37^{••},39]. Unlike IPEX patients, serum IgE levels were only mildly elevated in one patient and normal in the other [36,37^{••}].

In addition to autoimmune features, both CD25-deficient patients had infectious complications suggestive of a more extensive defect in cellular immunity. Most prominent in both patients was early-onset, recurrent

cytomegalovirus pneumonitis although persistent thrush, candidal esophagitis, chronic gastroenteritis and Epstein–Barr virus infection were also seen [36,37^{••}]. One patient failed to reject an allogeneic skin graft [38]. Owing to these ‘severe combined immunodeficiency-like’ features, one patient was successfully transplanted, following cytoreduction, with bone marrow from a matched sibling and has done well [38,39].

Immunophenotyping demonstrated either normal or modestly decreased T-lymphocyte numbers (particularly $CD4^{+}$ T cells). T-cell proliferative responses to mitogens and anti-CD3 stimulation were markedly decreased (below 20% of the control) but could be restored with high-dose interleukin-2 or interleukin-15 [36,37^{••}]. Evaluation of the T_{REG} compartment in human CD25 deficiency was limited, showing only that the percentage of FOXP3⁺ cells in the $CD4^{+}$ T cell population was comparable to a normal control, but slightly lower than that reported in the literature for other normal individuals (around 4% compared with 6–7% for other studies) [9,37^{••},40]. As T_{REG} cells from CD25-deficient patients lack the major cell surface marker used to differentiate and isolate intact T_{REG} , it is extremely difficult to assess their suppressive function in typical in-vitro assays. It is therefore unknown whether the $CD4^{+}$ FOXP3⁺ T_{REG} from these patients have normal suppressive capacity.

Insights gained from mice lacking CD25 suggest that T_{REG} development is normal in the thymus and that $CD4^{+}$ FOXP3⁺ T cells from these animals have normal suppressive function *in vitro*. There is, however, a defect in the survival, maintenance and competitive fitness of mature T_{REG} cells which is thought to be the etiology of the immune dysregulation observed [41,42]. Future efforts to assess the maturity of the $CD4^{+}$ FOXP3⁺ T_{REG} pool in patients will hopefully help to determine whether a similar mechanism is at play in humans.

STAT5b deficiency

STAT5b is a member of the signal transducer and activator of transcription family of proteins which regulate gene transcription in response to various cytokines and growth factors. It plays a particularly important role in T cells, where it is a key mediator of interleukin-2-induced responses. Like other signal transducer and activator of transcription proteins, STAT5b is present as a monomer in the cytoplasm of quiescent cells. It is recruited to the activated interleukin-2 receptor, where it is phosphorylated by receptor-associated tyrosine kinases. The phosphorylated subunits dimerize through their SH2 domains, translocate to the nucleus and bind DNA to regulate gene transcription [43].

Deficiency of STAT5b (Online Mendelian Inheritance in Man #245590) causes a rare autosomal recessive

disorder reported in only a handful of individuals [44–46]. The most notable clinical feature of the syndrome is dwarfism associated with a normal serum growth hormone level, but very low insulin-like growth factor-1 levels [44,46]. Other physical features include a prominent forehead, saddle nose and a high-pitched voice (see Table 1). Most patients also have a marked immunodeficiency, with recurrent varicella virus, herpes virus and *Pneumocystis jiroveci* infections, suggesting a defect in natural killer or cytotoxic T cells [44,46]. Immunophenotyping of affected patients has shown low $\gamma\delta$ T-cell and natural killer cell numbers and modest T-cell lymphopenia with a normal CD4/CD8 ratio [44,47**].

In addition to frank immunodeficiency, most patients who lack functional STAT5b also have symptoms suggestive of immune dysregulation including chronic, early-onset diarrhea, eczema and lymphocytic interstitial pneumonitis [44–46]. Murine models have demonstrated that STAT5b is a critical transducer of interleukin-2-mediated signals required to sustain FOXP3 expression in T_{REG} and to maintain T_{REG} cells themselves [48,49*]. Mice lacking STAT5b have a significant reduction in the number of FOXP3⁺ T_{REG} cells in thymus and spleen, and as a consequence develop splenomegaly and have a marked increase of activated T cells in the periphery [48,50].

To evaluate the effect of STAT5b deficiency on T_{REG} cells in humans, two patients have been studied: one with a homozygous missense mutation (A630P) in the SH2 domain of STAT5b and the second with a homozygous nonsense mutation (R152X). Both result in markedly reduced or absent STAT5b protein expression [44,47**]. In both cases, patients had significantly fewer CD4⁺CD25^{high} cells than normal individuals and the cells that were present expressed much less FOXP3 than normal CD4⁺CD25^{high} T_{REG} [44,47**]. Consequently, isolated CD4⁺CD25^{high} T cells from a STAT5b-deficient patient had no suppressive activity on either autologous or allogeneic responder T cells in an *in vitro* suppression assay [47**]. Decreased CD25 expression (around 20% of normal) on the patient's T cells in response to activation was shown to be a direct consequence of STAT5b deficiency, and is thought to synergize with the underlying STAT5b mutation to effectively abrogate interleukin-2 signals required for the maintenance of FOXP3 expression and T_{REG} function [47**]. Interestingly, signaling pathways required for interleukin-2-induced expression of other effector molecules such as perforin remained intact in STAT5b-deficient T cells [47**].

Autoimmune polyendocrinopathy, candidiasis and ectodermal dystrophy syndrome

APECED (Online Mendelian Inheritance in Man #240300) is an autosomal recessive disorder characterized

by systemic autoimmunity affecting primarily the endocrine organs including parathyroid glands, adrenal glands and pancreas [2,51]. Hypoparathyroidism, adrenal insufficiency and chronic mucocutaneous candidiasis typically characterize the syndrome, but patients may also have type 1 diabetes, gonadal failure and pernicious anemia secondary to atrophy of gastric parietal cells (see Table 1) [2,51].

APECED is caused by mutations in the autoimmune regulator AIRE – a transcription factor that plays a role in ectopic expression of tissue-specific antigens on thymic medullary epithelial cells. In mice, AIRE-mediated self-antigen expression in the thymus has been shown to play a significant role in negative selection of autoreactive T-cell clones, thus preventing their escape into the periphery where they may cause autoimmunity [52,53]. The mechanism by which AIRE causes expression of a wide range of tissue-specific gene products is unknown, but recent evidence suggests that it may do so through regulating large-scale access to chromatin.

Since naturally arising T_{REG} cells are also thymically derived, it was hypothesized that AIRE may also play a role in generation of T_{REG}, but various studies in AIRE-deficient mice failed to show a significant deficit in bulk T_{REG} numbers or function [52,54,55]. Recent investigations, using a transgenic mouse model with a mono-specific T-cell receptor, demonstrated that expression of the transgenic antigen (hemagglutinin) on AIRE⁺ medullary epithelial cells resulted in effective generation of antigen-specific CD4⁺CD25⁺FOXP3⁺ T_{REG} that had potent suppressive activity *in vitro* [56**]. These data suggest that the autoimmunity seen in AIRE^{-/-} mice is the result of defective negative selection of autoreactive effector T cells as well as defective generation of antigen-specific T_{REG}.

As the pace and severity of autoimmune disease in AIRE^{-/-} mice is relatively mild compared with that seen in patients with APECED, it was hypothesized that in humans AIRE may play a more significant role in the generation and function of T_{REG}. To address this possibility, two studies have evaluated patients with APECED by flow cytometry and quantitative real-time polymerase chain reaction for the presence of FOXP3⁺ T_{REG} cells in the peripheral blood. In each study, patients were found to have a decreased percentage of CD4⁺CD25^{high} T_{REG} cells within the CD4⁺ T-cell population. In addition, CD4⁺CD25^{high} T cells from patients expressed less FOXP3 than similar cells from normal controls [57**,58]. Coincident with the decreased FOXP3 expression in the CD4⁺CD25^{high} cells, isolated T_{REG} from APECED patients had a decreased ability to suppress proliferation of effector T cells in response to

anti-CD3 or mitogen (phytohemagglutinin) [57^{••}]. These data support a role for AIRE in generation of functional T_{REG} in humans.

Wiskott–Aldrich syndrome

The Wiskott–Aldrich syndrome (WAS) (Online Mendelian Inheritance in Man #301000) is a rare X-linked disorder characterized by thrombocytopenia, small platelets, eczema, recurrent infections, immunodeficiency, and a high incidence of autoimmune disease and malignancies (see Table 1). It is caused by mutations of the WAS protein (WASP) gene [59]. WASP has five well-defined functional domains and is a key regulator of actin polymerization in hematopoietic cells, where it is involved in signaling, cell locomotion and immune synapse formation.

As 40–70% of WAS patients develop autoimmune disease [60,61], several recent studies have focused on the quantity and quality of T_{REG} cells in both murine models and patients lacking functional WASP [62^{••}–64^{••}]. In mice, WASP appears to play no role in thymic T_{REG} production, but is required for peripheral T_{REG} expansion and survival [63^{••},64^{••}]. Specifically, WASP^{−/−} T_{REG}, compared with wild-type T_{REG}, showed decreased competitive fitness in three independent in-vivo murine models, including skewing of T_{REG} in heterozygous female carriers, progressive loss of WASP^{−/−} T_{REG} in recipients of chimeric bone marrow and in scurfy mice that received WASP^{−/−} T_{REG} via adoptive transfer [63^{••}]. While WASP deficiency in humans is not associated with a significant decrease in the percentage of T_{REG} in the peripheral blood, the function of T_{REG} was found to be consistently reduced in WAS patients, as demonstrated by the inability to suppress proliferation of effector T cells.

Consistent with the observations in mice, a strong selective advantage for WASP⁺ T_{REG} *in vivo* was observed in a patient with a revertant mutation leading to the expression of WASP in a significant percentage of newly generated T_{REG}. It appeared that in this patient the presence of a population of T_{REG} expressing WASP correlated with decreased autoimmune disease activity and improved clinical condition [63^{••}].

Conclusion

In recent years, identification of the gene defects in a handful of clinical syndromes with congenital systemic autoimmunity has led to the definition of a new class of PIDDs in which the defect is in the regulatory compartment of the immune system. The study of these syndromes has helped to expand our understanding of the development and function of T_{REG} cells in the normal immune system. Lessons learned about T_{REG} from these newer syndromes are now being applied to older, more

traditional PIDDs that have a high incidence of autoimmunity to define new roles for old friends in the rapidly expanding field of T_{REG} biology. Undoubtedly, new relationships will be identified that may affect how we treat both PIDDs and autoimmune diseases.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 589).

- 1 Sneller MC, Dale JK, Straus SE. Autoimmune lymphoproliferative syndrome. *Curr Opin Rheumatol* 2003; 15:417–421.
- 2 Halonen M, Eskelin P, Myhre AG, *et al*. AIRE mutations and human leukocyte antigen genotypes as determinants of the autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy phenotype. *J Clin Endocrinol Metab* 2002; 87:2568–2574.
- 3 Gambineri E, Torgerson TR, Ochs HD. Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX), a syndrome of systemic autoimmunity caused by mutations of FOXP3, a critical regulator of T-cell homeostasis. *Curr Opin Rheumatol* 2003; 15:430–435.
- 4 Powell BR, Buist NR, Stenzel P. An X-linked syndrome of diarrhea, polyendocrinopathy, and fatal infection in infancy. *J Pediatr* 1982; 100:731–737.
- 5 Russell WL, Russell LB, Gower JS. Exceptional inheritance of a sex-linked gene in the mouse explained on the basis that the X/O sex-chromosome constitution is female. *Proc Natl Acad Sci U S A* 1959; 45:554–560.
- 6 Levy-Lahad E, Wildin RS. Neonatal diabetes mellitus, enteropathy, thrombocytopenia, and endocrinopathy: further evidence for an X-linked lethal syndrome. *J Pediatr* 2001; 138:577–580.
- 7 Wildin RS, Smyk-Pearson S, Filipovich AH. Clinical and molecular features of the immunodysregulation, polyendocrinopathy, enteropathy, X linked (IPEX) syndrome. *J Med Genet* 2002; 39:537–545.
- 8 Kobayashi I, Imamura K, Yamada M, *et al*. A 75-kD autoantigen recognized by sera from patients with X-linked autoimmune enteropathy associated with nephropathy. *Clin Exp Immunol* 1998; 111:527–531.
- 9 Gavin MA, Torgerson TR, Houston E, *et al*. Single-cell analysis of normal and FOXP3-mutant human T cells: FOXP3 expression without regulatory T cell development. *Proc Natl Acad Sci U S A* 2006; 103:6659–6664.
- 10 Allan SE, Crome SQ, Crellin NK, Passerini L, *et al*. Activation-induced FOXP3
- in human T effector cells does not suppress proliferation or cytokine production. *Int Immunol* 2007; 19:345–354.

A thorough demonstration of the activation-induced expression of FOXP3 in human T cells – an important consideration for anyone evaluating T cells in humans.

- 11 Pillai V, Ortega SB, Wang CK, Karandikar NJ. Transient regulatory T-cells: a state attained by all activated human T-cells. *Clin Immunol* 2007; 123:18–29.
 - 12 Wang J, Ioan-Facsinay A, Van der Voort EI, *et al*. Transient expression of FOXP3 in human activated nonregulatory CD4⁺ T cells. *Eur J Immunol* 2007; 37:129–138.
 - 13 Carlsson P, Mahlapuu M. Forkhead transcription factors: key players in development and metabolism. *Dev Biol* 2002; 250:1–23.
 - 14 Schubert LA, Jeffery E, Zhang Y, *et al*. Scurfin (FOXP3) acts as a repressor of transcription and regulates T cell activation. *J Biol Chem* 2001; 276:37672–37679.
 - 15 Bettelli E, Dastrange M, Oukka M. Foxp3 interacts with nuclear factor of activated T cells and NF-kappa B to repress cytokine gene expression and effector functions of T helper cells. *Proc Natl Acad Sci U S A* 2005; 102:5138–5143.
 - 16 Wu Y, Borde M, Heissmeyer V, *et al*. FOXP3 controls regulatory T cell function
 - through cooperation with NFAT. *Cell* 2006; 126:375–387.
- Demonstration of a direct interaction of FOXP3 with NFAT proteins on the interleukin-2 promoter where FOXP3 suppresses nuclear factor of activated T cells-mediated gene transcription.
- 17 Zheng Y, Josefowicz SZ, Kas A, *et al*. Genome-wide analysis of Foxp3 target
 - genes in developing and mature regulatory T cells. *Nature* 2007; 445:936–940.
- A combination of gene expression arrays with ChIP-on-ChIP analysis that compares endogenous T_{REG} with effector T cells and links DNA binding of FOXP3 to gene expression at a large number of promoters.

- 18 Marson A, Kretschmer K, Frampton GM, *et al.* Foxp3 occupancy and regulation of key target genes during T-cell stimulation. *Nature* 2007; 445:931–935.
A genome-wide comparison of gene expression and promoter occupancy by Foxp3 in Foxp3⁺ vs. Foxp3[−] T-cell hybridoma lines.
- 19 Lopes JE, Torgerson TR, Schubert LA, *et al.* Analysis of FOXP3 reveals multiple domains required for its function as a transcriptional repressor. *J Immunol* 2006; 177:3133–3142.
Structure/function analysis of the human FOXP3 protein demonstrating the functional effect of mutations identified in patients with IPEX.
- 20 Ono M, Yaguchi H, Ohkura N, *et al.* Foxp3 controls regulatory T-cell function by interacting with AML1/Runx1. *Nature* 2007; 446:685–689.
Demonstration of an interaction between AML1/Runx1 and Foxp3 that is critical for gene repression by Foxp3 at the interleukin-2 promoter.
- 21 Godfrey VL, Rouse BT, Wilkinson JE. Transplantation of T cell-mediated, lymphoreticular disease from the scurfy (sf) mouse. *Am J Pathol* 1994; 145:281–286.
- 22 Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4⁺CD25⁺ regulatory T cells. *Nat Immunol* 2003; 4:330–336.
- 23 Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 2003; 299:1057–1061.
- 24 Khattri R, Cox T, Yasayko SA, Ramsdell F. An essential role for Scurfin in CD4⁺CD25⁺ T regulatory cells. *Nat Immunol* 2003; 4:337–342.
- 25 Yagi H, Nomura T, Nakamura K, *et al.* Crucial role of FOXP3 in the development and function of human CD25⁺CD4⁺ regulatory T cells. *Int Immunol* 2004; 16:1643–1656.
- 26 Lin W, Haribhai D, Relland LM, *et al.* Regulatory T cell development in the absence of functional Foxp3. *Nat Immunol* 2007; 8:359–368.
Demonstration that T cells destined to become T_{REG}, but unable to express Foxp3, develop a phenotype and gene expression profile suggestive of effector/cytotoxic function.
- 27 Gavin MA, Rasmussen JP, Fontenot JD, *et al.* Foxp3-dependent programme of regulatory T-cell differentiation. *Nature* 2007; 445:771–775.
Demonstration that T cells destined to become T_{REG}, but unable to express Foxp3, still express markers that typically identify the T_{REG} lineage (i.e. CD25, CTLA-4), but are unable to suppress *in vitro*.
- 28 Ferguson PJ, Blanton SH, Saulsbury FT, *et al.* Manifestations and linkage analysis in X-linked autoimmunity-immunodeficiency syndrome. *Am J Med Genet* 2000; 90:390–397.
- 29 Coenen JJ, Koenen HJ, van Rijssen E, *et al.* Rapamycin, not cyclosporine, permits thymic generation and peripheral preservation of CD4⁺CD25⁺ FoxP3⁺ T cells. *Bone Marrow Transplant* 2007; 39:537–545.
- 30 Bindl L, Torgerson T, Perroni L, *et al.* Successful use of the new immune-suppressor sirolimus in IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome). *J Pediatr* 2005; 147:256–259.
- 31 Battaglia M, Stabilini A, Migliavacca B, *et al.* Rapamycin promotes expansion of functional CD4⁺CD25⁺FOXP3⁺ regulatory T cells of both healthy subjects and type 1 diabetic patients. *J Immunol* 2006; 177:8338–8347.
Demonstration that rapamycin preferentially allows T_{REG} to expand relative to effector T cells.
- 32 Mazzolari E, Forino C, Fontana M, *et al.* A new case of IPEX receiving bone marrow transplantation. *Bone Marrow Transplant* 2005; 35:1033–1034.
- 33 Lucas KG, Ungar D, Comito M, *et al.* Submyeloablative cord blood transplantation corrects clinical defects seen in IPEX syndrome. *Bone Marrow Transplant* 2007; 39:55–56.
- 34 Rao A, Kamani N, Filipovich A, *et al.* Successful bone marrow transplantation for IPEX syndrome after reduced-intensity conditioning. *Blood* 2007; 109:383–385.
A case series of four successful bone marrow transplants using a reduced-intensity conditioning regimen in patients with IPEX.
- 35 Willerford DM, Chen J, Ferry JA, *et al.* Interleukin-2 receptor alpha chain regulates the size and content of the peripheral lymphoid compartment. *Immunity* 1995; 3:521–530.
- 36 Sharfe N, Dadi HK, Shahar M, *et al.* Human immune disorder arising from mutation of the alpha chain of the interleukin-2 receptor. *Proc Natl Acad Sci U S A* 1997; 94:3168–3171.
- 37 Caudy AA, Reddy ST, Chatila T, *et al.* CD25 deficiency causes an immune dysregulation, polyendocrinopathy, enteropathy, X-linked-like syndrome, and defective IL-10 expression from CD4 lymphocytes. *J Allergy Clin Immunol* 2007; 119:482–487.
Description of the IPEX-like clinical presentation of the second patient described with CD25 deficiency.
- 38 Roifman CM. Human IL-2 receptor alpha chain deficiency. *Pediatr Res* 2000; 48:6–11.
- 39 Aoki CA, Roifman CM, Lian ZX, *et al.* IL-2 receptor alpha deficiency and features of primary biliary cirrhosis. *J Autoimmun* 2006; 27:50–53.
- 40 Roncador G, Brown PJ, Maestre L, *et al.* Analysis of FOXP3 protein expression in human CD4⁺CD25⁺ regulatory T cells at the single-cell level. *Eur J Immunol* 2005; 35:1681–1691.
- 41 D'Cruz LM, Klein L. Development and function of agonist-induced CD25⁺Foxp3⁺ regulatory T cells in the absence of interleukin 2 signaling. *Nat Immunol* 2005; 6:1152–1159.
- 42 Fontenot JD, Rasmussen JP, Gavin MA, Rudensky AY. A function for interleukin 2 in Foxp3-expressing regulatory T cells. *Nat Immunol* 2005; 6:1142–1151.
- 43 Kim HP, Imbert J, Leonard WJ. Both integrated and differential regulation of components of the IL-2/IL-2 receptor system. *Cytokine Growth Factor Rev* 2006; 17:349–366.
- 44 Bernasconi A, Marino R, Ribas A, *et al.* Characterization of immunodeficiency in a patient with growth hormone insensitivity secondary to a novel STAT5b gene mutation. *Pediatrics* 2006; 118:e1584–e1592.
- 45 Hwa V, Little B, Adiyaman P, *et al.* Severe growth hormone insensitivity resulting from total absence of signal transducer and activator of transcription 5b. *J Clin Endocrinol Metab* 2005; 90:4260–4266.
- 46 Kofoed EM, Hwa V, Little B, *et al.* Growth hormone insensitivity associated with a STAT5b mutation. *N Engl J Med* 2003; 349:1139–1147.
- 47 Cohen AC, Nadeau KC, Tu W, *et al.* Cutting edge: decreased accumulation and regulatory function of CD4⁺CD25^{high} T cells in human STAT5b deficiency. *J Immunol* 2006; 177:2770–2774.
Description of the regulatory T-cell defects in a patient with STAT5b deficiency.
- 48 Burchill MA, Yang J, Vogtenhuber C, *et al.* IL-2 receptor beta-dependent STAT5 activation is required for the development of Foxp3⁺ regulatory T cells. *J Immunol* 2007; 178:280–290.
- 49 Yao Z, Kanno Y, Kerenyi M, *et al.* Nonredundant roles for Stat5a/b in directly regulating Foxp3. *Blood* 2007; 109:4368–4375.
Demonstration of the function of the two STAT5 isoforms in regulating Foxp3 expression in murine T cells. Helps to explain the mechanism by which STAT5b deficiency causes T_{REG} defects.
- 50 Antov A, Yang L, Vig M, *et al.* Essential role for STAT5 signaling in CD25⁺CD4⁺ regulatory T cell homeostasis and the maintenance of self-tolerance. *J Immunol* 2003; 171:3435–3441.
- 51 Perheentupa J. Autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy. *J Clin Endocrinol Metab* 2006; 91:2843–2850.
- 52 Liston A, Lesage S, Wilson J, *et al.* Aire regulates negative selection of organ-specific T cells. *Nat Immunol* 2003; 4:350–354.
- 53 Anderson MS, Venanzi ES, Klein L, *et al.* Projection of an immunological self shadow within the thymus by the aire protein. *Science* 2002; 298:1395–1401.
- 54 Kuroda N, Mitani T, Takeda N, *et al.* Development of autoimmunity against transcriptionally unexpressed target antigen in the thymus of Aire-deficient mice. *J Immunol* 2005; 174:1862–1870.
- 55 Anderson MS, Venanzi ES, Chen Z, *et al.* The cellular mechanism of Aire control of T cell tolerance. *Immunity* 2005; 23:227–239.
- 56 Aschenbrenner K, D'Cruz LM, Vollmann EH, *et al.* Selection of Foxp3⁺ regulatory T cells specific for self antigen expressed and presented by Aire⁺ medullary thymic epithelial cells. *Nat Immunol* 2007; 8:351–358.
Demonstration that antigen expression on Aire-expressing thymic medullary epithelial cells leads to generation of antigen-specific CD4⁺CD25⁺FOXP3⁺ T_{REG} cells with in-vitro suppressive capacity.
- 57 Kekalainen E, Tuovinen H, Joensuu J, *et al.* A defect of regulatory T cells in patients with autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy. *J Immunol* 2007; 178:1208–1215.
Demonstration that patients with APECED have decreased numbers of T_{REG} cells that also have decreased suppressive function *in vitro*.
- 58 Ryan KR, Lawson CA, Lorenzi AR, *et al.* CD4⁺CD25⁺ T-regulatory cells are decreased in patients with autoimmune polyendocrinopathy candidiasis ectodermal dystrophy. *J Allergy Clin Immunol* 2005; 116:1158–1159.
- 59 Ochs HD, Thrasher AJ. The Wiskott–Aldrich syndrome. *J Allergy Clin Immunol* 2006; 117:725–738; quiz 739.
- 60 Sullivan KE, Mullen CA, Blaese RM, Winkelstein JA. A multiinstitutional survey of the Wiskott–Aldrich syndrome. *J Pediatr* 1994; 125 (6 Pt 1):876–885.
- 61 Dupuis-Girod S, Medioni J, Haddad E, *et al.* Autoimmunity in Wiskott–Aldrich syndrome: risk factors, clinical features, and outcome in a single-center cohort of 55 patients. *Pediatrics* 2003; 111 (5 Pt 1):e622–e627.
- 62 Maillard MH, Cotta-de-Almeida V, Takeshima F, *et al.* The Wiskott–Aldrich syndrome protein is required for the function of CD4⁺CD25⁺Foxp3⁺ regulatory T cells. *J Exp Med* 2007; 204:381–391.
Demonstration that WASP[−] T_{REG} have decreased suppressive capacity compared with wild-type T_{REG}.

63 Humblet-Baron S, Sather B, Anover S, *et al.* Wiskott–Aldrich syndrome protein is required for regulatory T cell homeostasis. *J Clin Invest* 2007; 117:407–418. Demonstration that WASP[−] T_{REG} have decreased competitive fitness compared with wild-type T_{REG}. Report of a selective advantage for WASP-expressing T_{REG} cells in a WAS patient with a reversion mutation that restores WASP expression.

64 Marangoni F, Trifari S, Scaramuzza S, *et al.* WASP regulates suppressor activity of human and murine CD4⁺CD25⁺FOXP3⁺ natural regulatory T cells. *J Exp Med* 2007; 204:369–380. Demonstration that WASP deficiency in both mice and humans leads to functional defects in T_{REG} cells.