

Defect of regulatory T cells in patients with Omenn syndrome

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Background: Omenn syndrome (OS) is an autosomal-recessive disorder characterized by severe immunodeficiency and T-cell-mediated autoimmunity. The disease is caused by hypomorphic mutations in recombination-activating genes that hamper the process of Variable (V) Diversity (D) Joining (J) recombination, leading to the generation of autoreactive T cells. We have previously shown that in OS the expression of autoimmune regulator, a key factor governing central tolerance, is markedly reduced.

Objective: Here, we have addressed the role of peripheral tolerance in the disease pathogenesis.

Methods: We have analyzed forkhead box protein P3 (FOXP3) expression in peripheral blood T cells of 4 patients with OS and in lymphoid organs of 8 patients with OS and have tested the suppressive activity of sorted CD4⁺ CD25^{high} peripheral blood T cells in 2 of these patients.

Results: We have observed that CD4⁺ CD25^{high} T cells isolated *ex vivo* from patients with OS failed to suppress proliferation of autologous or allogenic CD4⁺ responder T cells. Moreover, despite individual variability in the fraction of circulating FOXP3⁺ CD4 cells in patients with OS, the immunohistochemical analysis of FOXP3 expression in lymph nodes and thymus of patients with OS demonstrated a severe reduction of this cell subset compared with control tissues.

Conclusion: Overall, these results suggest a defect of regulatory T cells in OS leading to a breakdown of peripheral tolerance, which may actively concur to the development of autoimmune manifestations in the disease. (*J Allergy Clin Immunol* 2010;125:209-16.)

Key words: Immunodeficiency, V(D)J recombination, Omenn syndrome, regulatory T cells, FOXP3, anergy and tolerance, thymus and the development of T lymphocytes

Omenn syndrome (OS) is a peculiar form of combined immunodeficiency presenting with early-onset generalized erythrodermia, failure to thrive, alopecia, lymphadenopathy, hepatosplenomegaly, and intractable diarrhea.¹ The immunologic phenotype of OS is characterized by a normal to increased number of autologous T lymphocytes that express activation markers, whereas circulating B cells are usually low or absent. Immunoglobulin serum levels are very low, with the notable exception of IgE levels, which are often increased.^{2,3}

OS is most frequently associated with hypomorphic mutations in the recombination-activating genes (RAGs), which impair but do not completely abolish V(D)J recombination process, leading to the generation of only a few productive antigen receptor gene rearrangements. As a consequence, T-cell repertoire is highly restricted.⁴⁻⁷ More recently, additional gene defects that severely reduce but do not completely ablate T-cell differentiation have been shown to account for OS in a proportion of patients.⁸ Whatever the molecular defects, oligoclonal and activated T cells infiltrate various organs, including skin, gut, spleen, and liver, resulting in profound tissue damage.⁹⁻¹¹ We have previously reported that the thymus from patients with OS is markedly abnormal, with lack of corticomedullary demarcation and absence of Hassall bodies. In addition, the expression of autoimmune regulator (AIRE) and AIRE-dependent tissue-restricted antigens is severely reduced.¹² On the basis of these findings, it has been hypothesized that loss of central tolerance may contribute to the immunopathology of OS. More recently, the lack of invariant natural killer T cells (iNKT) has been proposed to contribute to the immunopathology of OS.¹³ However, the possibility that defects in other mechanisms of tolerance might be affected in OS has not been tested so far. Peripheral dominant control of autoreactive T cells is primarily mediated by natural occurring CD4⁺ CD25⁺ forkhead box protein P3 (FOXP3)⁺ regulatory T (Treg) cells.¹⁴⁻¹⁷ Indeed, several human autoimmune diseases have been associated with alterations in Treg-cell functions.¹⁸ The thymic development of this cell population is critically dependent on the expression of the transcription repressor gene *FOXP3*,¹⁹ which also represents a valuable marker to identify both thymic and peripheral Treg cells.²⁰ In human beings, there is evidence that in the thymic medulla Treg cells are generated from thymocytes on high-affinity T-cell receptor-peptide-MHC-mediated interaction with a subset of activated dendritic cells, driving these cells to acquire proper regulatory function.²¹ Although FOXP3 is a marker for human Treg cells, it cannot be

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Abbreviations used

AIRE: Autoimmune regulator
 FOXP3: Forkhead box protein P3
 HD: Healthy donor
 OS: Omenn syndrome
 RAG: Recombination-activating gene
 Treg: Regulatory T

considered specific for this population because it can also be expressed, along with CD25, by activated CD4⁺ cells.²²⁻²⁴

In the current study, we have analyzed FOXP3 expression in peripheral blood T cells of 4 patients with OS and in lymphoid organs of 8 patients with OS and have tested the suppressive activity of sorted CD4⁺ CD25^{high} CD127^{low/-} peripheral blood T cells in 2 of these patients. Our results provide for the first time evidence of impaired development and function of Treg cells in OS, implying that both central and peripheral tolerance are compromised in this disease.

METHODS**Patients**

Eight patients with RAG1 defects and 1 patient with a RAG2 defect previously described (patient 5¹²) were included in this study. The clinical, immunologic, and molecular features of the patients, consistent with OS, are outlined in Tables I and II. Briefly, patient 1 was a boy who presented in the first days of life with erythrodermia, lymphadenopathy, spleen, and liver enlargement. Laboratory analysis showed leukocytosis (59×10^3 cells/ μ L) with marked eosinophilia (40×10^3 cells/ μ L) and the presence of T and natural killer cells but an absence of B cells. In patient 2, a diagnosis of OS was made at 2 months of age after observation of erythrodermia and lymphadenopathy. Immunologic studies showed hypogammaglobulinemia, high levels of IgE (>5000 IU/mL), and impaired lymphocyte proliferative response to mitogens (anti-CD3, 6000 counts per minute; PHA, 2000 cpm). Patients 3 and 4 were previously described.¹³ Patient 3 presented at the age of 5 months with diarrhea and failure to thrive. Cow's milk protein intolerance was suspected, but a dairy-free diet remained ineffective. In the following weeks, the child developed interstitial pneumonia and dermatitis. Laboratory analysis revealed lymphopenia ($1.2 \text{ cells} \times 10^3$ cells/ μ L), a low number of T cells (CD3, 47%), a relative increase of natural killer cells (42%), and an absence of B cells. Maternal engraftment was undetectable by HLA chimerism analysis. In patient 4, clinical manifestations of OS were observed at 2 weeks of life. In particular, exudative erythrodermia, cervical lymphadenopathy, and hepatosplenomegaly were detected. Blood testing showed agammaglobulinemia and circulating T cells expressing activation markers. All patients were compound-heterozygous for RAG1 gene as described in Tables I and II.

Blood samples and inguinal lymph node biopsies were obtained from the patients during the clinical setting, whereas control reactive lymph nodes were obtained from patients with unrelated nonimmunologic diseases. OS thymus (patient -5) was retrieved during postmortem examination within 36 hours of death,¹² and control normal human thymus tissues were obtained anonymously from an infant with no known immunologic abnormalities during heart surgery, according to the protocol approved by the Institutional Review Board of the Spedali Civili, Brescia, Italy.

Fluorescence-activated cell sorting analysis

After PBMC purification by standard density gradient technique, cells were stained with anti-CD4 PerCP-Cy5.5-conjugated mAb (BD Pharmingen, San Diego, Calif) in combination with 1 of the following antihuman phycoerythrin-conjugated mAbs: CD25 (clone BC96; Biolegend, San Diego, Calif), CCR7 (R&D Systems, Minneapolis, Minn), and CD45RA

and HLA-DR (BD Pharmingen). After 20 minutes of room temperature incubation, cells were washed, and the intracellular staining for antihuman FOXP3 was performed by using an Alexa 488-conjugated mAb (clone 259D; Biolegend) according to the manufacturer's protocol. Samples were acquired the same day of the staining using a FACSCalibur flow cytometer (Becton Dickinson) and analyzed by using FlowJo software (TreeStar Inc, Ashland, Ore).

In vitro suppression assays

CD4⁺CD25^{high}CD127^{low/-} Treg cells²⁵ and CD4⁺CD25⁻CD127⁺ responder T cells from patients with OS and age-matched healthy controls were isolated from PBMCs by fluorescence-activated cell sorting. In both cases, the purity was $\geq 95\%$. Suppression assays were performed as follows: 5×10^4 responder T cells were stimulated in U-bottom 96-well plates with 1 μ g/mL soluble anti-CD3 mAbs (Orthoclone OKT3; Janssen-Cilag, Milan, Italy) in the presence of an equal number of allogeneic accessory cells (APCs), in a final volume of 200 μ L complete medium. CD4⁺CD25^{high}CD127^{low/-} Treg cells were added at a ratio of 1:0.5 (responder/suppressor). Accessory cells were obtained by immunomagnetic depletion of CD3⁺ cells by using CD3-coated beads (Miltenyi, Bergisch Gladbach, Germany) from PBMCs of healthy controls, followed by 30 minutes of treatment with Mitomycin C (40 μ g/mL; Sigma Aldrich, St Louis, Mo). After 72 hours of coculture, 50 μ L culture supernatant was collected to test for IFN- γ production by ELISA, and cells were pulsed for 16 hours with 1 μ Ci per well [³H]thymidine (GE Healthcare, Little Chalfont, UK). Cells were harvested and counted in a scintillation counter. Considering the limited number of cells plated per well and the individual variability in the proliferation rate, which may affect the results, the assay reliability was carefully ensured by testing the suppressive activity, in either autologous or allogeneic settings, of a cohort of healthy donors (HDs; n = 8/9).

Immunohistochemical studies

Four- μ m-thick sections of lymph nodes and thymus tissues were taken from formalin-fixed, paraffin-embedded blocks and subjected to routine hematoxylin and eosin staining and immunohistochemical analysis. Briefly, sections were dewaxed and rehydrated and endogenous peroxidase activity blocked with 0.3% H₂O₂ in methanol for 15 minutes. Heat induced epitope retrieval was performed by treatment in 1.0 mmol/L EDTA buffer pH 8.0 in a thermostatic bath for 40 minutes at 98 °C. Sections were then cooled, washed in a TRIS-base buffer at pH 7.4, preincubated in blocking buffer containing 5% normal goat serum in TRIS-HCl for 5 minutes, and incubated for 1 hour with primary antibody (rat antihuman Foxp3, 1:200; eBioscience, San Diego, Calif) in TRIS/1% BSA. Sections were then washed again before incubation for 30 minutes with the appropriate secondary antibody (biotinylated rabbit antirat, 1:100; Vector, Burlingame, Calif). Reactivity was revealed by incubation in streptavidin-horseradish peroxidase and diaminobenzidine DAB (DAKO Cytomation, Glostrup, Denmark) and slides counterstained with hematoxylin. Images were acquired by an Olympus DP70 camera mounted on an Olympus Bx60 microscope using Cell^F imaging software (Soft Imaging System GmbH).

Real-time PCR

RNA was purified from whole frozen thymus using the guanidinium thiocyanate-phenol-chloroform method according to the instruction manual (RNAwiz; Ambion Inc, Austin, Tex). One microgram of deoxyribonuclease-treated total RNA was used to synthesize the first strand of cDNA with the GeneAmp RNA PCR kit (Applied Biosystems, Foster City, Calif). For real-time PCR analysis, Assays-on-Demand products (20 $\times$) and TaqMan Master Mix (2 $\times$) from Applied Biosystems were used to amplify FOXP3 and GAPDH genes according to the manufacturer's instructions. Reactions were run on an ABI PRISM 7700 Sequence Detection System (Applied Biosystems). Expression levels of FOXP3 were normalized to GAPDH levels in each sample.

TABLE I. Clinical and genetic features of patients with OS

	Pt-1	Pt-2	Pt-3	Pt-4	HD (n = 10) median (range)
Age at onset	1 wk	2 mo	5 mo	2 wk	
Skin lesions	++++	++	+	++++	
Hepatosplenomegaly	++++	++++	+	++++	
Colitis	-	-	+++	+/-	
Lymphadenopathy	+++	+++	-	+	
Eosinophilia	+++	++	+	+	
Infectious episodes	CMV at 1 wk	Neonatal sepsis	-	<i>Klebsiella, Candida</i> at 2 mo	
CD3 (cells/ μ L)	10,491	8,607	187	763	4,215 (2,014-6,954)
CD4 (cells/ μ L)	10109	5748	75	696	2882 (1611-4389)
CD25, % of CD4 cells	80.6	67.4	62.1	10.2	9.65 (6.02-15.6)
FOXP3, % of CD4 cells	4.8	4.5	32.8	1.9	6.58 (4.77-6.96)
HLA-DR, % of CD4 cells	84.5	92.6	19.6	51.5	10.4 (4.06-25.8)
CD19 (cells/ μ L)	2	2	<0.1	<0.1	1,053 (411-2,972)
IgM (mg/dL)	8	7	<5	25	
IgG (mg/dL)	†	†	†	<66	
IgE (UI/mL)	—	>5,000	<2	—	(n.v. <20 IU/mL)
Protein change*	R142X/M458fr	P85fr/E722 K	R332X/R561H	G392E/L732fr	

CMV, Cytomegalovirus; n.v., normal value; Pt, patient.

*All patients were compound-heterozygous for *RAG1* gene mutations.

†Patient receiving intravenous immunoglobulin at the time of the analysis.

TABLE II. Summary of the pathologic features and FOXP3 expression of the inguinal lymph nodes

Patient	Mutation protein change	Lymph node diameter (mm)	Lymphoid cellularity	T cells	B cells	No. of FOXP3 ⁺ (cells/mm ²) $\pm$ SD
Pt-1	R142X; M458fr	6	D	+	+/-	58 $\pm$ 4.03
Pt-2	P85fr; E722K	12	D	+	+/-	100 $\pm$ 7.24
Pt-3	R332X; R561H	NA	NA	NA	NA	NA
Pt-4	G392E; L732fr	10	ND	+	0	163 $\pm$ 3.36
Pt-5	R229Q*	5	D	+/-	0	17 $\pm$ 1.14
Pt-6	R396C; Y912C	5	D	+/-	0	10 $\pm$ 0.70
Pt-7	R396H; I538fr	28	D	+	0	4 $\pm$ 0.83
Pt-8	R561H	6	ND	+	+/- (rare PF)	497 $\pm$ 4.03
Pt-9	R759C; N968 K	20	ND	++	0	344 $\pm$ 7.69
Control-1		12	RL	++	++ (PF, SF)	996 $\pm$ 20.96
Control-2		15	RL	++	++ (PF, SF)	899 $\pm$ 14.02
Control-3		10	RL	++	++ (PF, SF)	820 $\pm$ 13.45
Control-4		10	RL	++	++ (PF, SF)	783 $\pm$ 15.89

D, Depleted; NA, not analyzed; ND, nondepleted; PF/SF, primary/secondary B follicles; Pt, patient with OS; RL, reactive lymph node.

0, Undetectable; +/-, rare; +/+, moderate/numerous cells.

*Mutation in *RAG2* gene. All the other patients were compound-heterozygous for *RAG1* gene mutations.

Statistics

A 2-tailed Mann Whitney *U* test (nonparametric analysis) was used for statistical comparison of patients versus healthy controls. A *P* value less than .05 was considered significant.

RESULTS

Phenotypic characterization of FOXP3⁺ cells in the peripheral blood of patients with OS

We analyzed CD4⁺ CD25⁺ FOXP3⁺ T cells from the peripheral blood of 4 patients with OS caused by mutations in the *RAG1* gene

(Table I). The percentage of FOXP3⁺ cells among the CD4⁺ CD25⁺ cells were in the normal range for patients 1, 2, and 4, whereas in patient 3, this subset was markedly increased (Fig 1, A). Quantitative analysis of the absolute number of CD4⁺ FOXP3⁺ cells showed broad variability in these patients (Fig 1, B). Interestingly, the 2 patients (patients 1 and 2) displaying the highest number of circulating CD4⁺ FOXP3⁺ showed perinatal infections (Table I). However, there is evidence that FOXP3 expression is not restricted to Treg cells but is also detected on activated T lymphocytes.^{22,23} On the basis of this evidence, we have further characterized the phenotype of the FOXP3⁺ T-cell subset by

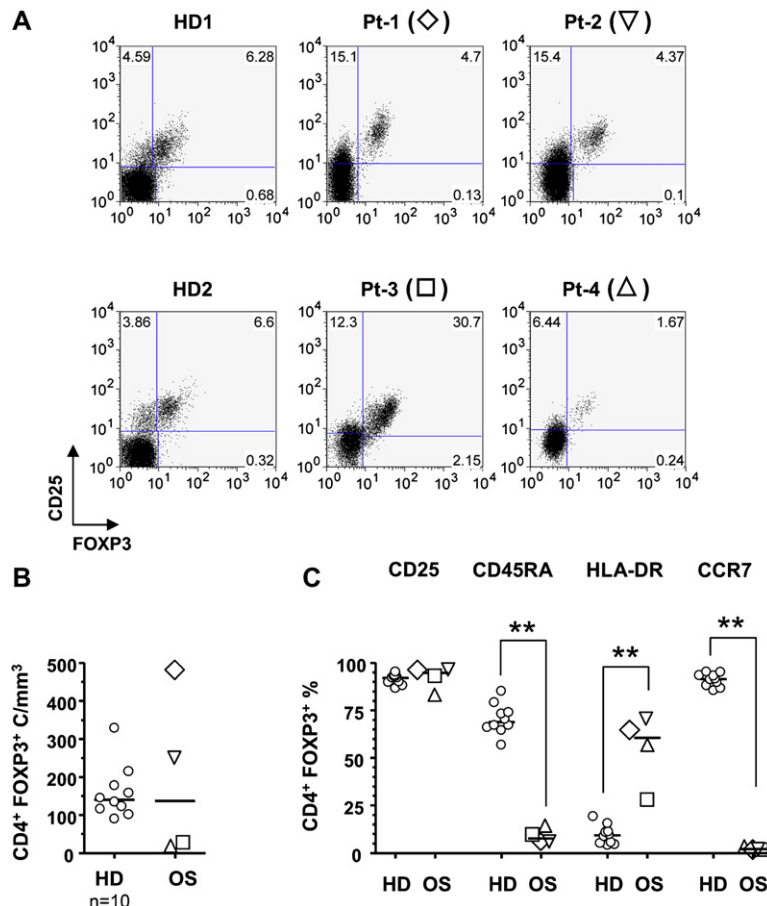


FIG 1. Phenotype of FOXP3⁺ Treg cells in patients (Pt) with OS. **A**, PBMCs from 4 patients with OS and 2 representative age-matched HDs were stained with anti-CD4, anti-CD25, and anti-FOXP3 mAbs. Results shown are gated on the CD4⁺ T cells. Quadrant markers were set based on staining with isotype control mAbs and numbers indicate the percentage of each subset within the CD4⁺ population. **B**, Absolute counts of circulating CD4⁺ FOXP3⁺ cells. **C**, Activation status of CD4⁺ FOXP3⁺ cells. The percentages of CD4⁺ FOXP3⁺ cells expressing CD25, CD45RA, HLA-DR, and CCR7 markers are reported. ***P* < .01.

studying expression of CD25, CD45RA, HLA-DR, and CCR7. The proportion of CD4⁺ FOXP3⁺ T cells expressing CD25 was elevated (Foxp3⁺/CD25⁺ > 90%) to a comparable extent in both patients with OS and age-matched HDs. However, whereas CD4⁺ FOXP3⁺ cells from healthy controls displayed a HLA-DR⁺ CCR7⁺ phenotype and most of them coexpressed CD45RA, the CD4⁺ FOXP3⁺ cells from patients with OS were CD45RA⁺ CCR7⁺ and expressed variable levels of HLA-DR, thus resembling the phenotype of activated memory T cells (Fig 1, C).

CD4⁺ CD25^{high} T cells from patients with OS exhibit impaired suppressive function *in vitro*

Next we tested the ability of CD25^{high} cells isolated from patients with OS to suppress proliferative responses. Because of the severity of the disease and the difficulties of obtaining adequate blood samples from patients with OS, CD4⁺ CD25^{high} CD127^{low/-} conventional regulatory²⁵ and CD4⁺ CD25^{high} CD127^{low/-} responder T cells were purified by cell sorting from PBMCs of 2 patients (patients 1 and 2) and activated with soluble anti-CD3 mAb in the presence of allogeneic accessory cells (Fig 2, A) in comparison with cells isolated from age-matched HDs.

Control Treg cells from HDs were unresponsive to anti-CD3–triggered stimulation and strongly suppressed both the proliferation range and the IFN- γ secretion of cocultured autologous responder T cells stimulated with anti-CD3 mAb (Fig 2, A). CD25^{high} Treg cells from patients with OS were also anergic. Although they appeared to exert no suppressive effect on the proliferation of CD4⁺ CD25⁺ cells (Fig 2, A), 1 obvious limitation in the interpretation of these data was that responder T cells from patients with OS failed to mount a robust proliferative response on stimulation with anti-CD3 mAb (Fig 2, A). To overcome this problem, the suppressive activity of CD4⁺ CD25^{high} CD127^{low/-} cells from patients with OS (or from healthy controls) was also tested in an allogeneic setting, in which third-party CD4⁺ CD25⁺ CD127⁺ T cells from healthy controls were used as responder T cells and were activated with anti-CD3 mAb. The assay reliability was preliminary validated testing a cohort of HDs. As shown in Fig 2, B, healthy control Treg cells consistently suppressed proliferation and IFN- γ secretion of allogeneic third-party responder T cells, with a percentage of inhibition ranging from 47 to 64.5. In contrast, no or markedly reduced suppressive activity was observed when responder T cells were activated in the presence of CD4⁺ CD25^{high} CD127^{low/-} cells isolated from patients with OS.

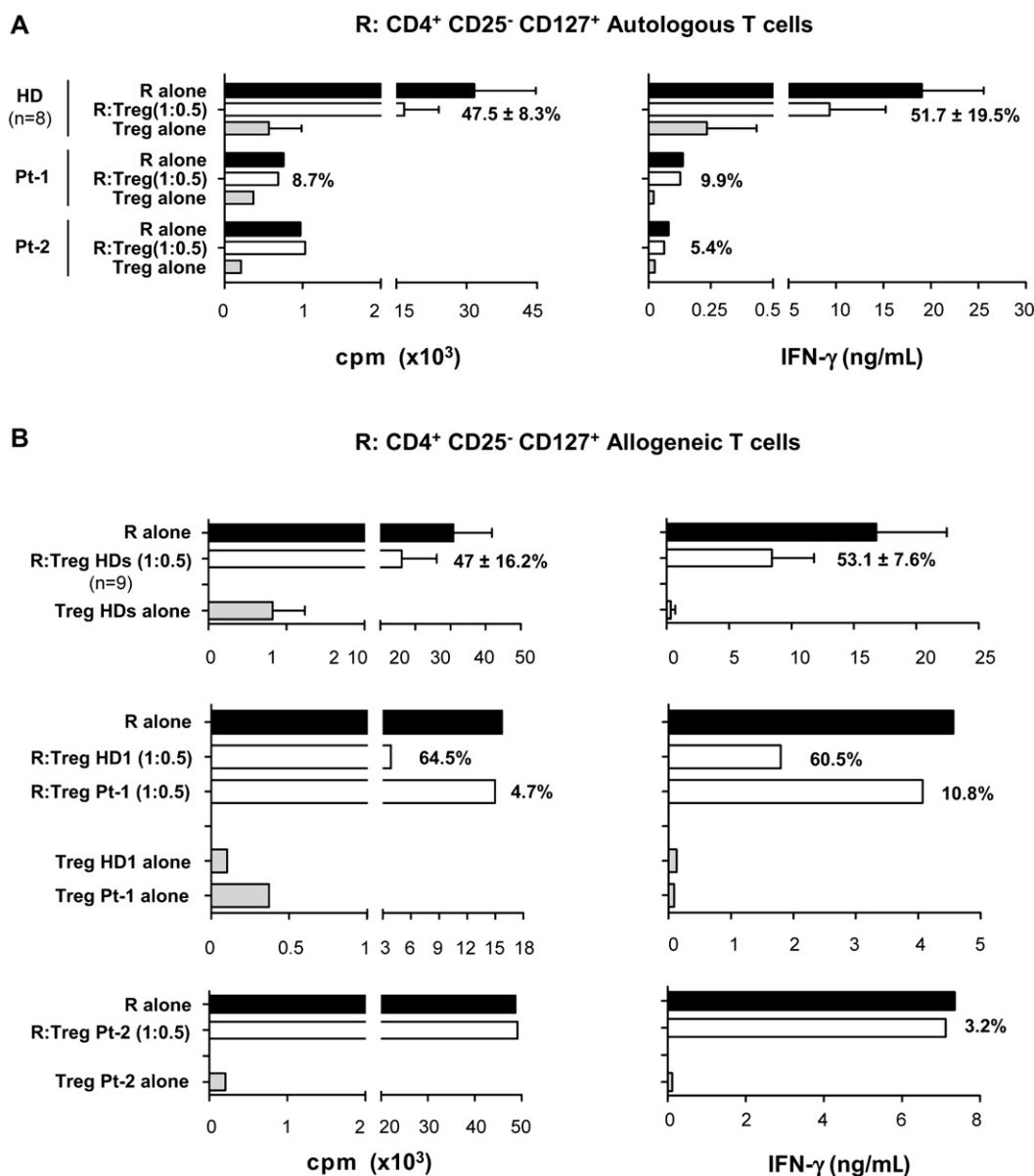


FIG 2. Suppressive activity of patients' CD4⁺ CD25^{high} Treg cells. The ability of freshly isolated CD4⁺ CD25^{high} CD127^{low} Treg cells from patients with OS and HDs to suppress proliferation of either autologous (A) or allogeneic (B) CD4⁺ CD25⁻ CD127⁺ responder T cells was assessed. Treg cells were added to activated responder cells (R) at 0.5:1 ratio, and proliferation was evaluated by [³H]thymidine incorporation (see Methods for details). In parallel, the IFN-γ secretion was measured in culture supernatants. Percentages indicate inhibition of proliferation or cytokine secretion (average ± SD in case multiple experiments testing different HDs).

Lymph nodes and thymus from patients with OS have reduced number of FOXP3⁺ T cells

Morphologic evaluation of lymph nodes from 8 patients with OS revealed severe architectural alterations with expansion of the paracortical area and dermatopathic changes, along with marked depletion of the lymphoid cell population with lack of B follicles and variable number of T cells (referred to as “depleted lymph nodes” in Table II).⁹ Partial recognizable architectural organization with rare tiny B follicles and the presence of a moderate number of T lymphocytes were found in some of the OS lymph nodes (referred to as “nondepleted lymph nodes” in Table II).

Immunohistochemical evaluation of FOXP3 expression showed pronounced reduction of FOXP3⁺ cells in the lymph nodes of patients with OS (n = 8; average 149.1 ± 180.4 SDs FOXP3⁺ cells/mm²) with respect to controls (n = 4; average 874.5 ± 94.3 SDs FOXP3⁺ cells/mm²; Fig 3 and Table II; P < .001). Interestingly, a higher number of FOXP3⁺ cells/mm² were found in lymph nodes showing a nondepleted pattern (n = 3; average 334.7 ± 167.2 SDs FOXP3⁺ cells/mm²; Fig 3, B, and Table II) than in those with severe depletion of the lymphoid cell component (n = 5; average 37.8 ± 40.7 SDs FOXP3⁺ cells/mm²; Fig 3, C, and Table II; P < .05).

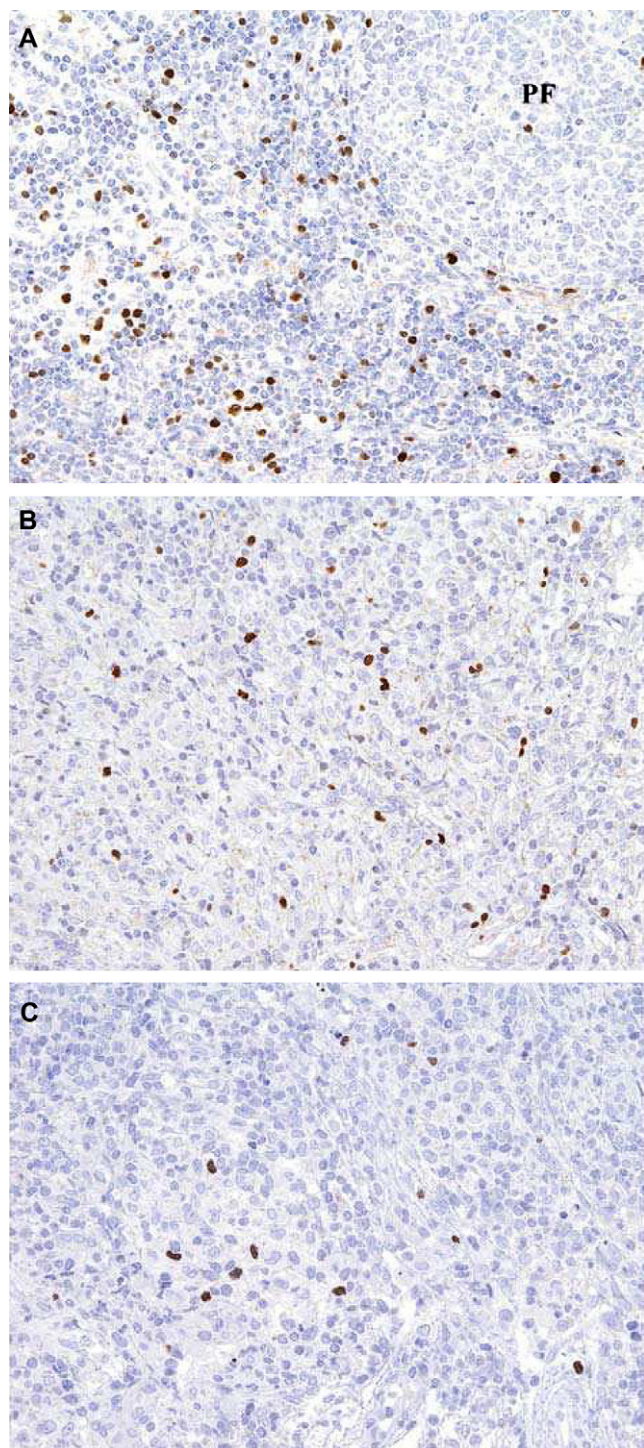


FIG 3. Histopathologic features and FOXP3 expression in the lymph nodes of patients with OS. Inguinal lymph nodes sections from an HD showed numerous FOXP3⁺ cells distributed within the T-cell areas (**A**). In contrast, lymph nodes sections from 2 representative biopsies from patients with OS revealed either a reduced number of FOXP3⁺ cells in the lymph nodes showing nondepleted features (patient 4; **B**) or a dramatic depletion of FOXP3⁺ cells in the lymph nodes with a depleted histopathologic pattern (patient 1; **C**). FOXP3⁺ cells, brown nuclear staining. All panels, original magnification $\times 20$.

To explore whether deficiency of Treg cells in the periphery might result from impaired thymic development, we investigated the presence of FOXP3⁺ cells in the thymic biopsy from 1 patient with OS lacking AIRE expression in the thymus (patient 5 in Table II)¹² and in the thymus from an age-matched nonimmunodeficient patient who underwent heart surgery. In normal thymus, FOXP3⁺ cells were predominantly localized in the thymic medulla around the Hassall bodies (Fig 4, A), as previously described.²¹ In contrast, the thymus of the patient with OS was markedly atrophic with no corticomedullary differentiation and showed a virtual absence of FOXP3⁺ cells (Fig 4, B). In keeping with this observation, the relative FOXP3 mRNA level, as assessed by real-time PCR analysis, was severely reduced in thymus from the patient with OS in comparison with the control biopsy, although slightly higher than the expression levels detected for the thymus of a patient with severe combined immunodeficiency (RAG1 heterozygous compound mutation¹²) whose genetic defect completely prevented the normal T-cell development (Fig 4, C).

DISCUSSION

In this article, we demonstrate that patients with OS have a variable number of circulating FOXP3⁺ T lymphocytes. However, at variance from what was observed in normal individuals, circulating FOXP3⁺ cells in patients with OS coexpress activation markers and fail to suppress proliferation of allogenic activated CD4⁺ T cells. OS is characterized by a profound impairment of T lymphocyte generation and abnormalities in the mechanisms that govern central tolerance. In this context, it is likely that circulating T cells from patients with OS include autoreactive T cells that are activated in the periphery on interaction with antigens and that hence may present increased expression of FOXP3.²³ A high level of FOXP3 has been recently associated with a newly defined subset of activated CD25^{high} CD45RA⁺ Treg cells, which would represent a late differentiation stage of resting thymus-derived Treg cells (FOXP3^{lo} CD45RA⁺ CD25^{dim}).²⁶ Although a subset of peripheral T cells in patients with OS shows a phenotype FOXP3^{high} CD45RA⁺ resembling activated Treg cells, this subpopulation lacks suppressive activity *in vitro*. We speculate that the abnormal T-cell receptor rearrangement and compensatory peripheral homeostatic proliferation affect the differentiation of functionally competent activated Treg cells. Furthermore, resting Treg cells (FOXP3^{lo} CD45RA⁺ CD25^{dim}) are generated in the thymus, mostly in the medulla and at the corticomedullary junction on recognition of self-antigens presented by a subset of mature activated dendritic cells.^{21,27} In contrast, we have shown that the thymus of patients with OS is virtually devoid of FOXP3⁺ cells. This defect adds to our previous demonstration of profound abnormalities of thymic architecture and lack of AIRE expression.¹² Interestingly, a role of AIRE in the generation of Treg cells is supported by studies performed in patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy, a monogenic autoimmune disorder caused by mutations in the *AIRE* gene. These patients display a severe depletion of CD25^{high} Treg cells within the CD4⁺ peripheral T-cell population that is associated with reduced FOXP3 protein expression.^{28,29} Whether the virtual lack of FOXP3⁺ cells in the thymus of patients with OS is secondary to impaired expression of AIRE or whether it results from broader abnormalities in thymic architecture and microenvironment remains to be defined. In keeping

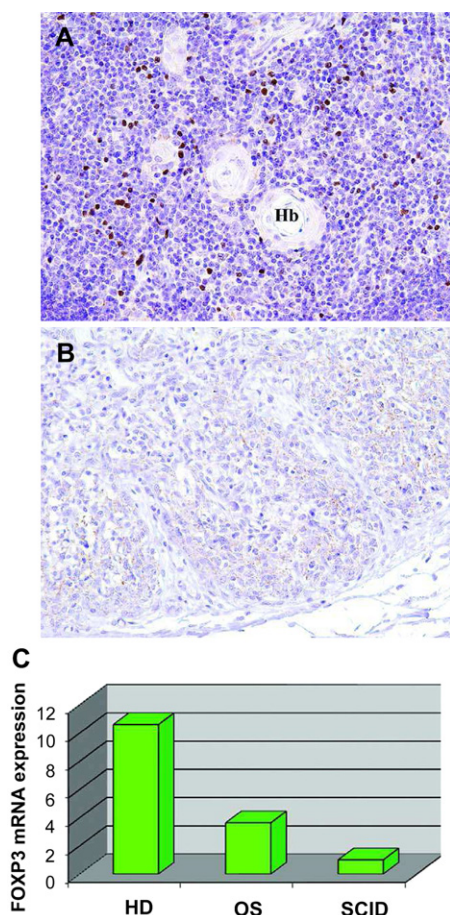


FIG 4. Histopathologic features and FOXP3 expression in the thymus of a patient with OS. Thymus sections from an HD showed numerous FOXP3⁺ cells distributed within the thymic medulla near the Hassall bodies (Hb; **A**). The thymic biopsy of the patient with OS (patient 5; **B**) did not show any evidence of FOXP3 protein expression. Real-time PCR analysis of cDNA prepared from RNA isolated from normal thymus and thymus of the same patient with OS confirmed the depletion of FOXP3 expression in OS (**C**). FOXP3 mRNA was undetectable in the patient with severe combined immunodeficiency (SCID). The level of FOXP3 mRNA, normalized for the expression level of GAPDH, was plotted as fold increase over those in the control. FOXP3⁺ cells, brown nuclear staining; **A** and **B**, original magnification $\times 20$.

with the demonstration of severe reduction in the number of FOXP3⁺ T cells in the thymus and with coexpression of activation markers by peripheral FOXP3⁺ cells, we have shown that CD4⁺ CD25^{high} lymphocytes isolated from these patients had a decreased capability to suppress *in vitro* proliferation of CD25⁺ responder T cells. These data strongly indicate that the FOXP3⁺ T cells present in patients with OS do not represent *bona fide* natural Treg cells but rather are *in vivo* activated T cells, as also reported in other experimental models. Furthermore, given the absence of CCR7 expression, the circulating FOXP3⁺ lymphocytes with the effector/memory phenotype (CD4⁺/CD45RO⁺/CCR7⁻) identified in patients with OS should be impaired in their capacity to reach lymph nodes and are predicted to home preferentially to peripheral tissues (skin, gut, and liver), thus possibly contributing to the profound tissue damage that is typically observed in this disease. This study was performed in a cohort of patients with OS caused by RAG defects. However, it is now clear that OS may also be caused by hypomorphic mutations in other

genes (eg, *DCLRE1C*, *IL7R*, *RMRP*, *IL2RG*, *ZAP70*, *LIG4*, *ADA*).⁸ Remarkably, it has recently been described similar thymic alterations associated with lack of AIRE expression, absence of Hassall corpuscles, and FOXP3⁺ T cells even in non-RAG-mutated patients with OS.³⁰ Therefore, overall these findings may provide the basis for a unifying mechanism of the immunopathology of this disease, involving impaired mechanisms of central tolerance and abnormalities in Treg development and function. The identification of common pathogenetic mechanisms among distinct inherited disorders might point at previously unrecognized targets for therapeutic interventions.

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Key messages

- Development and function of Treg cells are impaired in patients with OS.
- FOXP3 expression in the peripheral blood of patients with OS does not identify *bona fide* natural Treg cells, but rather is consistent with an *in vivo* T-cell activation process.

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