Reduced central tolerance in Omenn syndrome leads to immature self-reactive oligoclonal T cells

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Background: Omenn syndrome (OS) is characterized by a peculiar severe T-cell immune deficiency associated with autoimmunelike manifestations. Dysregulations of the central and peripheral immune tolerance, mediated by the protein autoimmune regulator (AIRE) and regulatory T cells, respectively, were proposed as possible mechanisms of this aberrant inflammatory process.

Objective: We studied mechanisms of central and peripheral tolerance in patients with OS and also examined the gene expression profile associated with OS features.

Methods: T-cell receptor diversity, DNA rearrangement, and the expression of AIRE and forkhead box P3 mRNA as well as the expression of regulatory T cells in cells obtained from patients with OS were studied. Characterization of gene expression in these cells was carried out by using the TaqMan Low-Density Array.

Results: Transcript expression of peripheral blood AIRE but not forkhead box P3 was reduced in patients with OS. The expression of natural killer T and regulatory T cells was normal, although the latter showed an abnormal CD4-negative population. Patients with OS have oligoclonal T cells with limited DNA recombination activity, including the presence of early but not late T-cell maturation events, regardless of the genetic defect underlying the syndrome. The transcriptional profile associated with OS features reveals significant changes in 25.5% of the tested genes compared with normal control.

Conclusion: Our findings suggest that T-cell oligoclonal expansion in OS emanates from an incomplete block before the maturation stage of negative selection, which may explain escape of autoreactive T cells from the thymus. Dysregulated genes in patients with OS are closely involved with self-tolerance and autoimmunity. (J Allergy Clin Immunol 2009;124:793-800.)

Key words: *Omenn syndrome, autoimmunity, immunodeficiency, SCID, AIRE, tolerance, Foxp3, Treg, oligoclones, TREC*

Omenn syndrome (OS) is characterized by severe susceptibility to infections, generalized scaly exudative erythroderma, enlarged lymphoid tissues, eosinophilia, and peripheral expansion of oligoclonal T cells.[1] Several genetic defects, which impair but do not completely abolish lymphocyte development, are known to cause OS, including hypomorphic mutations in recombinase-activating genes (RAGs) 1 and 2, Artemis, IL-7 receptor α, RNA component of mitochondrial RNA processing endoribonuclease (RMRP), DNA-ligase 4, adenosine deaminase, and IL-2 receptor γ.[2-8] Regardless of the underlying genetic defect, patients with OS have a restricted T-cell receptor (TCR) repertoire and exhibit peripheral expansion of self-reactive oligoclonal T cells.[9] These cells infiltrate and expand in different organs (eg, skin, gut, liver, and spleen). The suggested mechanism for this phenomenon is the possible inability of the thymus to delete these abnormal clones. Consequently, these autoreactive T cells expand in the periphery and cause tissue damage.[10]

Normally T-cell maturation in the thymus progresses through distinct stages that are defined phenotypically by the expression of TCR and the CD4 and CD8 coreceptors. On the basis of the expression of these cell surface markers, thymocytes can be classified as double-negative (DN; $CD4^-CD8^-$) thymocytes, double-positive (DP; $CD4^+CD8^+$) thymocytes, and single-positive (SP; $CD4^+$ or $CD8^+$) cells. The TCR maturation process consists of rearrangement of different DNA segments (Variable, Diversity, and Joining [VDJ]) to create a diverse repertoire. Thymocytes bearing TCR, with a high affinity for self-antigens presented by epithelial cells, are deleted.[11] The autoimmune regulator (AIRE) protein, which is expressed in different human lymphatic organs including thymus, spleen, and lymph node, and in a fraction of blood mononuclear cells,[12] was shown to play a critical role in eliminating these self-reactive T cells and in the maintenance of tolerance. Its deficiency leads to autoimmune disorders.[13] OS is considered a distinct inflammatory process that can be associated with genetically diverse leaky severe combined immunodeficiencies (SCIDs). It was suspected that reduced central tolerance might be a key factor in the pathogenesis of OS. Recently, it was shown that AIRE mRNA and protein levels are profoundly reduced in thymi of patients with OS and RAG mutations, suggesting AIRE participation in the development of autoimmune features in this condition.[14] The regulatory T (Treg) cell population ($CD4^+CD25^+$forkhead box P3 [$Foxp3]^+$)

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Abbreviations used
AIRE: Autoimmune regulator
DN: Double-negative
DP: Double-positive
Foxp3: Forkhead box P3
NKT: Natural killer T
OS: Omenn syndrome
RAG: Recombinase-activating gene
RMRP: RNA component of mitochondrial RNA processing endoribonuclease
RQ: Relative quantity
SCID: Severe combined immunodeficiency
SP: Single-positive
TCR: T-cell receptor
TLDA: TaqMan Low-Density Array
TREC: T-cell receptor excision circle
Treg: Regulatory T
VDJ: Variable, Diversity, and Joining

also ensures tolerance to autoantigens by actively suppressing autoreactive T-cell reactions in the periphery. The absence of these cells may underline autoimmune manifestations.[15,16] However, little is known about the role of peripheral tolerance in the pathogenesis of OS in human beings. Further, the presence of Treg cells in OS has not been carefully analyzed. To expand our understanding of self-tolerance in OS, we studied here T-cell diversity and the expression of AIRE as well as Foxp3 and CD25 in cells obtained from patients with OS. We have also characterized gene expression in these cells by using the TaqMan Low-Density Array (TLDA) (Applied Biosystems, Foster City, Calif).

METHODS

Immune workup and genetic analysis

Cells surface markers of PBMCs, lymphocyte proliferative responses to mitogens, the amount of signal joint T-cell receptor excision circles (TRECs), and sequencing RAG2 from genomic DNA of patients with OS and healthy controls were determined as previously described.[17] To estimate TREC copies, we compared the amplification cycle threshold (CT) value in a given sample with a standard curve obtained from PCRs performed with 10-fold serial dilutions of an internal standard. The amplification CT value for the internal standard was as follows: 10^6 copies, 20.8; 10^5 copies, 23.6; 10^4 copies, 27.7; 10^3 copies, 30.1; and 10^2 copies, 33.4. In 40 samples in which immunodeficiency was excluded, TREC copies were >400.

Quantitation of TCR genes

Representatives of specific TCR Vβ families were detected and quantified by using flow cytometry (EPICS ELITE, Beckman-Coulter, Fullerton, Calif) as previously described.[17] *TCR*-γ rearrangements were amplified by PCR according to the standardized Biomed 2 protocol.[18] For GeneScan analyses, Vγ primers were fluorescently labeled and used in PCRs as described in the Biomed 2 protocol. Fluorescence-labeled PCR amplificate (1 μL each) was added to a mixture of 12.5 μL deionized formamide and 0.5 μL GeneScan 500TM Rox internal lane standard (Applied Biosystems).

Cell isolation and analysis of Treg cells

PBMCs were obtained by density gradient centrifugation on a Histopaque 1077 (Sigma Aldrich, Israel). The mouse mAbs against human cell surface markers used were as follows: CD3–fluorescein isothiocyanate, CD4–fluorescein isothiocyanate, CD4-phycoerythrin, CD4-allophycocyanin, CD25-allophycocyanin (all obtained from BD Pharmingen, San Diego, Calif), CD25-phycoerythrin (Miltenyi Biotec, Bergisch Gladbach, Germany), and the 236A/E7 mouse anti-hFOXP3 mAbs (Bioscience). The isotype-matched control mAbs were all from BD Pharmingen. For detection of FOXP3, the cells were fixed/permeabilized by using the eBioscience FOXP3 staining buffer set according to the manufacturer's protocol. Cell samples were analyzed on a FACSCalibur by using the Cellquest software. The fluorescence-activated cell sorting data analysis was performed with the FlowJo 7.2.2 software (Tree Star, Ashland, Ky).

Real-time quantitative PCR assays

To isolate total RNA, 2 to 4×10^6 PBMCs were harvested by using the RNeasy kit (Qiagen, Valencia, Calif) according to the manufacturer's instructions. cDNA was made from 5 μg total RNA using the cDNA Archive kit (Applied Biosystems). Real-time PCR was performed by using an ABI 7900 Sequence Detection System (Applied Biosystems). Primers and probes for AIRE and FOXP3 were obtained as assays on demand from Applied Biosystems. For each analysis, transcription of the gene of interest was compared with transcription of the housekeeping gene B2 M that was amplified in parallel. The samples were run in triplicate, and fold change in transcription was calculated by using the $2^{-\Delta\Delta Ct}$ comparative method. The *t* test was used to assess whether the means of 2 groups were *statistically* different from each other. Differences were considered statistically significant at $P \leq .05$. In all experiments, cDNA was obtained from lymphocytes, and an equal amount of cDNA was used.

Analysis of human TCR gene rearrangement

Analysis was performed by using DNA extracted from the patient's PBMCs. Amplification reactions (25 μL) contained 0.5 μg genomic DNA, 12.5 μL TaqMan universal PCR master mix (Applied Biosystems, Foster City, Calif), and the appropriate primers and probes. PCR (2 minutes at 50°C followed by 95°C for 10 minutes, then 40 cycles at 95°C for 15 seconds and 60°C for 1 minute) was carried out in an ABI PRISM 7900 Sequence Detector *Taq*Man system (Applied Biosystems). Primers (900 nmol/L) and 6-carboxyfluorescein (FAM) probes (250 nmol/L) for PCR were used as previously described.[19] The number of TRECs in a given sample was estimated by comparing the CT value obtained with a standard curve obtained from PCRs performed with 10-fold serial dilutions of an internal standard provided by Dr Daniel Douek (Vaccine Research Center, National Institute of Allergy and Infectious Diseases, Bethesda, Md). The dilutions contained 10^2 to 10^6 copies of signal joint TRECs in triplicate. The number of copies of other recombinations of TCR genes in a given sample was relatively quantified.[20] Amplification of ribonuclease P (RNAseP) (Applied Biosystems) was used to verify the quantity and presence of genomic DNA. Age-matched normal individuals were used as controls. Each experiment was performed in triplicate.

mRNA gene analyses by quantitative RT-PCR

Predesigned TLDAs (96 TaqMan Gene Expression assay preconfigured in a 384-well format, part no. 4370573, microfluidic cards; Applied Biosystems), were used in a 2-step RT-PCR process with the ABI Prism 7900HT Sequence Detection System (Applied Biosystems) with a TLDA Upgrade (Applied Biosystems). Each cDNA sample (100 μL) was added to an equal volume of 2×TaqMan Universal PCR Master Mix (Applied Biosystems). After gentle mixing, the solution was transferred into a loading port on a TLDA card (Applied Biosystems). Each of the samples was analyzed in a single TLDA run with each of 2 TLDA experiments running on separate cards by using an Applied Biosystems 7900 HT Fast-Real-Time PCR System. Thermal cycler conditions were as follows: 2 minutes at 50°C, 10 minute at 94.5°C, and 30 seconds at 97°C, and 1 minute at 59.7°C for 40 cycles. Data were acquired according to the manufacturer's suggestions. For analysis, expression levels of target genes were normalized to β-glucuronidase. Gene expression values were then calculated based on the $\Delta\Delta C_t$ method, with data normalized to cDNA that was obtained from lymphocytes of an age-matched healthy control in which primary immunodeficiency was excluded by performing a basic immune workup.

Relative quantities (RQs) were determined by using the following equation:

$$RQ = 2^{-\Delta\Delta ct}$$

Only genes with reproducible amplification curves of duplicate determinations were analyzed and presented.

RESULTS

Characterization of the patient and his molecular defect

This male patient of Palestinian descent was born after a normal pregnancy and delivery to parents who were first-degree cousins. Two previous sisters had died during infancy from respiratory infections. At 3 months of age, he had failure to thrive, fever, cough, diarrhea, and respiratory distress. Physical examination revealed remarkable erythrodermia, alopecia, massive lymphadenopathy, and hepatosplenomegaly. Skin biopsy was consistent with OS and bronchoalveolar lavage was positive for *Pneumocystis jiroveci*.

The patient had undetectable levels of immunoglobulins and slightly reduced numbers of circulating lymphocytes (1320 cells/μL) with remarkable eosinophilia (2960 cells/μL). Flow-cytometric analysis showed a reduced number of $CD3^+$ cells (488 cells/μL) with 224 $CD4^+$ cells/μL and 224 $CD8^+$ cells/μL. There were 500 natural killer cells/μL but no detectable $CD19^+$ or $CD20^+$ B cells. Natural killer T (NKT) cells expressing CD3 and CD56 were 175/μL. Interestingly, 5% of the $CD3^+$ cells were positive to both CD4 and CD8 (DP), which suggests escape of immature T cells from the thymus before selection into SP cells. Response to mitogenic stimulation with PHA and anti-CD3 was profoundly reduced, and thymus output as determined by TREC copies was undetectable. The lack of circulating B cells, together with features of OS, was suggestive of RAG deficiency. Indeed, sequence analysis of the patient's RAG2 gene revealed a G1305 T homozygous mutation, which predicted a putative glycine to valine substitution at amino acid position 35. The parents were both heterozygous for this mutation and were asymptomatic. One healthy sibling was found to be homozygous for the wild-type allele (Fig 1, *A*).

Analysis of TCR repertoire in blood and skin

To define the patient's circulating T cells, we studied the repertoire of the $CD3^+$ lymphocytes (Fig 1, *B and C*). Almost the entire population of $CD3^+$ T cells was composed of the $CD3^+$ $V_ß20$ T-cell clone. A few other $CD3^+$ $V_ß$ families such as $V_ß3$, $V_ß5.1$, $V_ß7.2$, and $V_ß8$ had normal representation, whereas all other Vβ families were completely absent, indicating T-cell oligoclonality (Fig 1, *B*). To better understand the clonality of these cells in peripheral blood and their infiltration to the skin, we analyzed the TCRγ-chain gene rearrangement. The γ-chain gene, although typically not expressed on the cell surface of TCRαβ cells, remains rearranged and can be used as a clonality marker. We performed PCR analysis of TCRγ gene rearrangement in peripheral blood and skin samples from the patient as well as PBMCs from his mother. In the patient, a similarly restricted oligoclonal repertoire was demonstrated in PBMCs and skin in 3 Vγ rearranged genes. Moreover, the Vγ9/2 rearranged gene showed an identical oligoclonal pattern in the 2 patients' tissues examined, indicating clonal infiltration. In contrast, the Vγf1 rearranged gene was found in PBMCs but not in the skin, suggesting that this clone does not infiltrate the skin. Unlike the restriction pattern of the TCR Vγ gene rearrangement repertoire in the patient, PBMCs from his mother showed a polyclonal pattern in all 4 detected Vγ gene rearrangements. Careful cytogenetic analysis showed that only 3% of the patient's T cells were of maternal origin and thus could not account for the majority of expanded T cells in the blood or skin.

Origin of T-cell clones in OS

The TCR is created through a sequential order of recombination events between the different TCR genes (*TCRD* > *TCRG* > *TCRB* > *TCRA*). A sequential order also occurs within every particular gene. For example, in TCRD, Dδ2 to Dδ3 occurs earlier than the Dδ2 to Jδ1 rearrangement, and the Vδ to Jδ1 rearrangement occurs even later. Thus, different DNA segments are excised during this sequential process.[19] By assessing events of newly formed DNA recombination, we could predict the thymic origin of the patient's residual cells and compare them to other patients with SCID but no OS features, as clinically described in Table I. DNA obtained from mononuclear cells from our patient carried early recombination events but not late events (Dδ2 to Dδ3, Dδ2 to Jδ1, and not Vδ to Jδ1 rearrangements; Fig 2, *A*). This was in contrast with DNA obtained from residual T cells of 4 RAG2 (non-OS)–deficient patients who had no DNA recombination events at all.

To define whether the T-cell maturation defect and associated peripheral T-cell characterization described in our index patient was present in other patients with OS, yet with different underlying genetic defects, we studied DNA recombination events in patients with a mutation in RMRP with or without the OS phenotype. Only the RMRP-deficient patient with OS features had expression of early but not late DNA recombination events (Dδ2 to Dδ3, Dδ2 to Jδ1, and not Vδ to Jδ1 rearrangements; Fig 2, *B*), similar to the patient with OS with RAG deficiency.

Analysis of immune regulation mechanisms

Although thymic expression of AIRE appears reduced in the thymus of OS, its peripheral expression during similar circumstances remains unknown.[12] We studied the expression of AIRE and FOX3 in circulating mononuclear cells. RQ PCR studies revealed that the expression of AIRE transcript compared with the healthy control was significantly reduced ($P < .01$), whereas the expression of Foxp3 transcripts was similar to that in the healthy control (Fig 3, *A*). Next, enumeration of CD3, CD4, CD25, and intracellular FOXP3 by fluorescence-activated cell sorting analysis was performed. Unstimulated freshly isolated PBMCs stained with CD25 and Foxp3 antibodies on live $CD4^+$ T cells showed normal Foxp3 expression (4.6% of total gated cells; Fig 3, *B, middle*), similar to the healthy control (4.9% of total gated cells; Fig 3, *B, right*). Interestingly, freshly isolated PBMCs stained with CD4 and Foxp3 antibodies on live $CD3^+$ T cells revealed that of the positive CD3 and Foxp3 cells, 56% were $CD4^-$, whereas 44% were $CD4^+$ cells (Fig 3, *C, middle*). This was in contrast with a healthy control, who had almost 100% CD4, Foxp3, and $CD3^+$ cells (Fig 3, *C, right*). These findings may suggest an aberrant distribution of Treg cells between CD4 and possibly CD8 cells in the patient's blood.

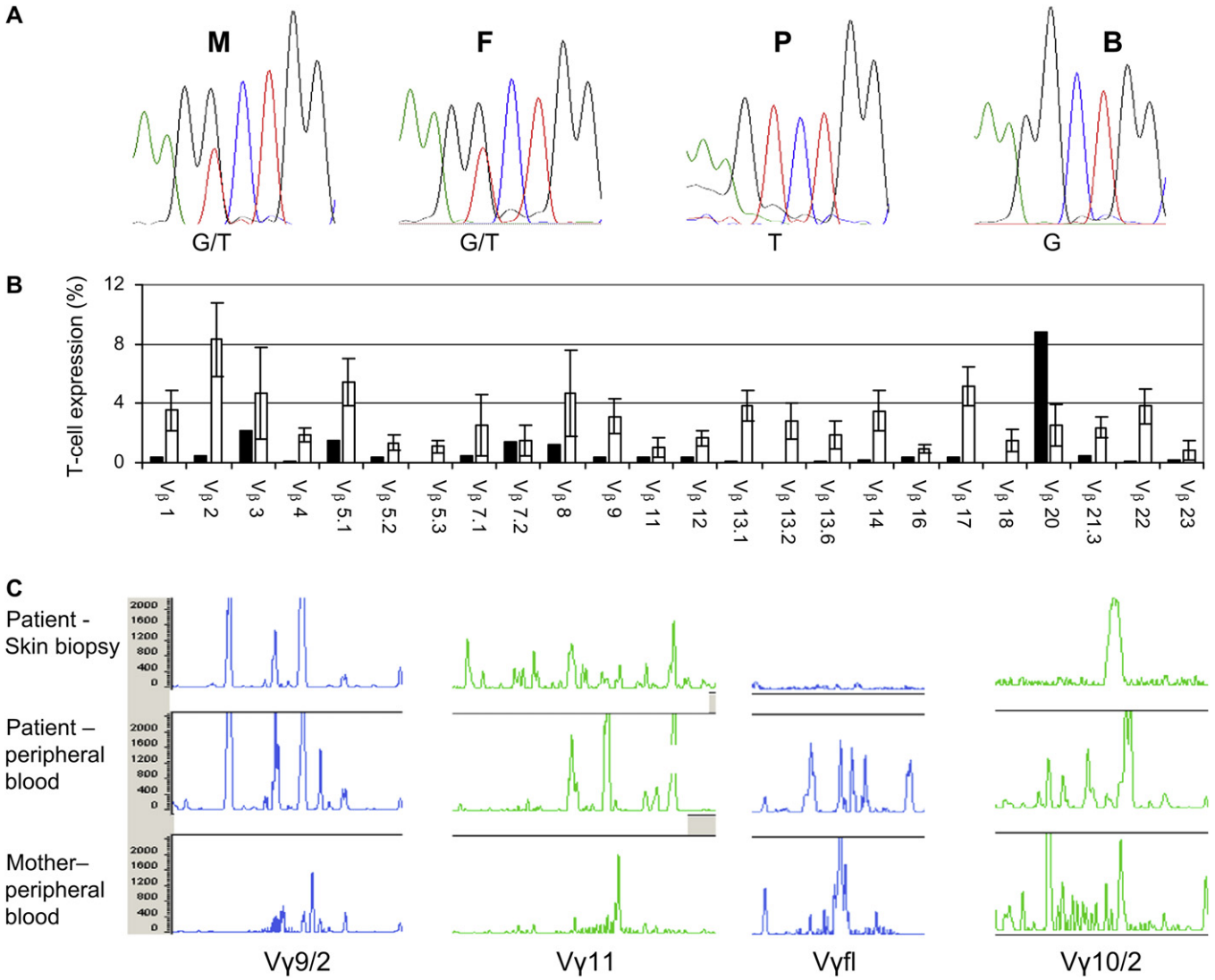


FIG 1. A, Sequence analysis of mutated region of RAG2 in patient *(P)*, parents (*M,* mother; *F,* father), and healthy sibling *(B)*. **B,** Relative expression of the various Vβ families in the patient's PBMCs *(black bar)* compared with normal controls. **C,** TCRγ spectratyping of fluorescence-labeled PCR products using 4 consensus Vγ primers (vg9/2, vg11, vgf1, vg10/2) for the characterization of the patient's (PBMCs and skin) and mother's (PBMCs) TCRγ.

Gene expression in OS circulating T cells

To better understand the gene transcriptional profiles associated with OS features, 90 genes related to the immune system were examined by TLDA. Results were normalized to β-glucuronidase, which was found in our experiment to be the most stable reference gene, and were compared with an age-matched healthy control. Twenty-three of 90 (25.5%) genes showed an expression level of more than 2-fold increase or decrease compared with control samples (Table II; $P < .05$, unpaired t test). Of these, the expression of 15 genes (16.6%) was downregulated (by a factor of 2-21.7, values of 0.5-0.04, respectively; Table II), and 8 genes (8.8%) were upregulated (by a factor of 2-7.1). Interestingly, several genes that are known to be involved in immune regulation and autoimmunity were found to be markedly upregulated (eg, IL-10, CCR2, FAS ligand, and IL-2 receptor α) or downregulated (CCR7, IL-17, IL-12, CD28, CD40 ligand, IL-1, CD40, and CD80; Table II).

DISCUSSION

OS is believed to be caused by impaired T-cell differentiation and abnormal self-tolerance. RAGs 1 and 2 and artemis encode proteins that are critical for VDJ recombination, and hypomorphic mutations in these genes, which allow some VDJ recombination, result in OS.[21] In addition, defects in other genes that are not involved in the VDJ recombination process also cause OS, suggesting that abnormal thymic microenvironment is important in the pathogenesis of OS.[22] Thus, a limited number of thymocytes formed, which are improperly selected in the thymus, leak into the circulation and eventually act as autoreactive T cells.

Thymic expression of AIRE is important for the cross-talk between the developing thymocytes and stromal cells and was suggested to be important in the process of central tolerance.[23] Indeed, AIRE mRNA and protein levels were found to be profoundly reduced in thymi of RAG-defective patients with OS.[14] Here we show that PBMCs of a patient with the OS phenotype

TABLE I. Immune workup and genetic defects in patients with SCID with/without Omenn phenotype

Patient no.	Genetic defect	Omenn/SCID	Lymph (× $10^3/\mu L$)	CD3 (× $10^3/\mu L$)	CD4 (× $10^3/\mu L$)	CD8 (× $10^3/\mu L$)	CD19 (× $10^3/\mu L$)	CD16/56 (× $10^3/\mu L$)
1	RAG2	Omenn	1320	488	224	224	0	500
2	RAG2	SCID	600	240	150	90	0	200
3	RAG2	SCID	576	109	100	9	0	432
4	RAG2	SCID	290	8	3	5	0	170
5	RAG2	SCID	110	39	15	8	0	46
6	RMRP	Omenn	820	237	133	142	475	47
7	RMRP	SCID	850	105	51	20	480	229

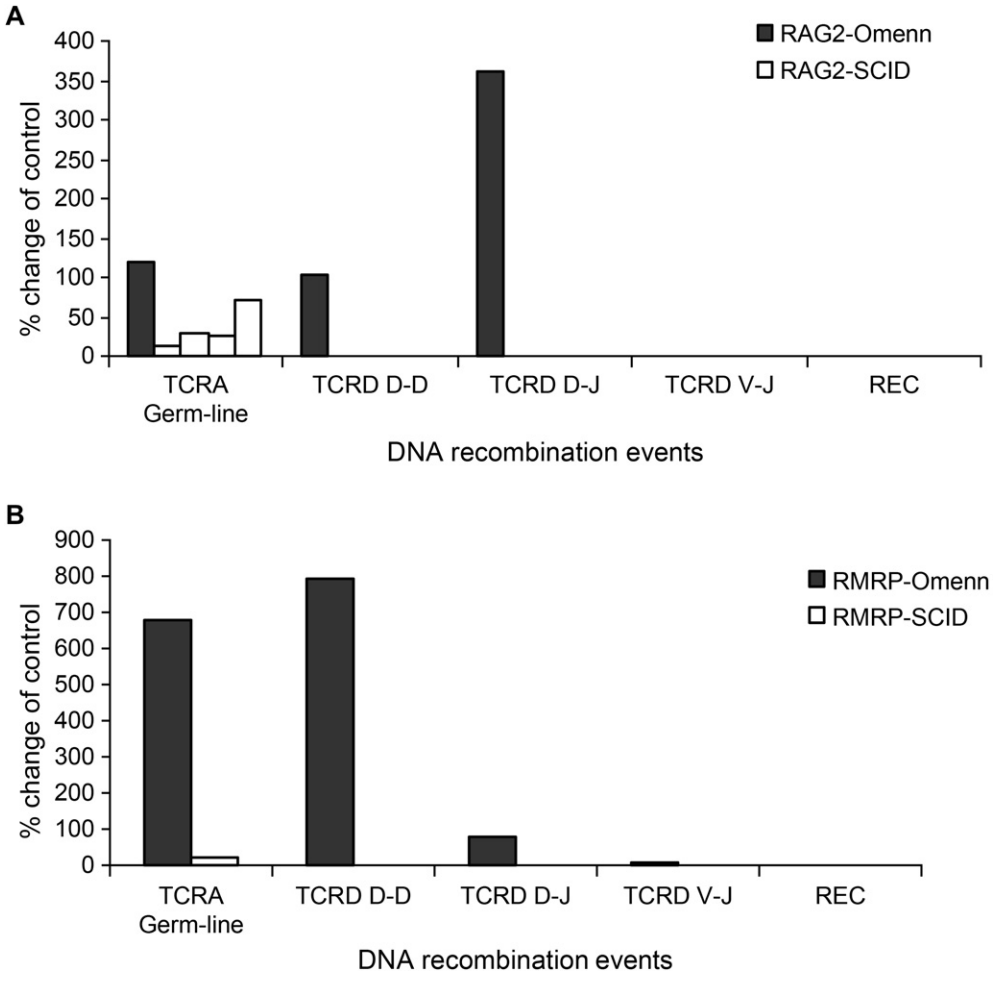


FIG 2. DNA was purified from PBMCs obtained from RAG2 SCID **(A)** and RMRP SCID **(B)** with *(black bar)* or without *(white bar)* OS features to determine sequential DNA recombination events of TCR. All experiments were performed in triplicates. *REC,* Receptor excision circles.

express low AIRE transcripts. The correlation of this finding to abnormal thymic expression of AIRE and its contribution to abnormal T-cell differentiation and negative selection remain to be clarified. We also tried to gain more insight into the thymic process of T-cell differentiation in patients with OS by analyzing sequential events of DNA recombination. These were known to correlate with the transition of DN cells to SP cells.[19] The presence of early but not late DNA recombination events was found in our patient, suggesting a defect in early T-cell maturation. Moreover, a similar pattern was found in another patient with OS who carried an RMRP mutation, but was not observed in 5 patients with SCID with no OS features. These novel observations indicate that patients with OS have a restricted pattern of DNA recombination in their released self-reactive cells. Furthermore, on the basis of the sequential order of DNA TCR recombination events and its correlation to the transition from DN to SP cells,[19] we could speculate that the leaky nature of the thymus in patients with OS occurred slightly before or during the stage of negative selection. During this maturation step, AIRE protein was shown to have a pivotal function. This may also partially explain the increased number of DP cells that probably leak from the thymus before they become SP cells. We assumed that although most rearrangements were blocked, as we have shown, some clones may have leaked while maturing, in the process forming autoreactive cells.

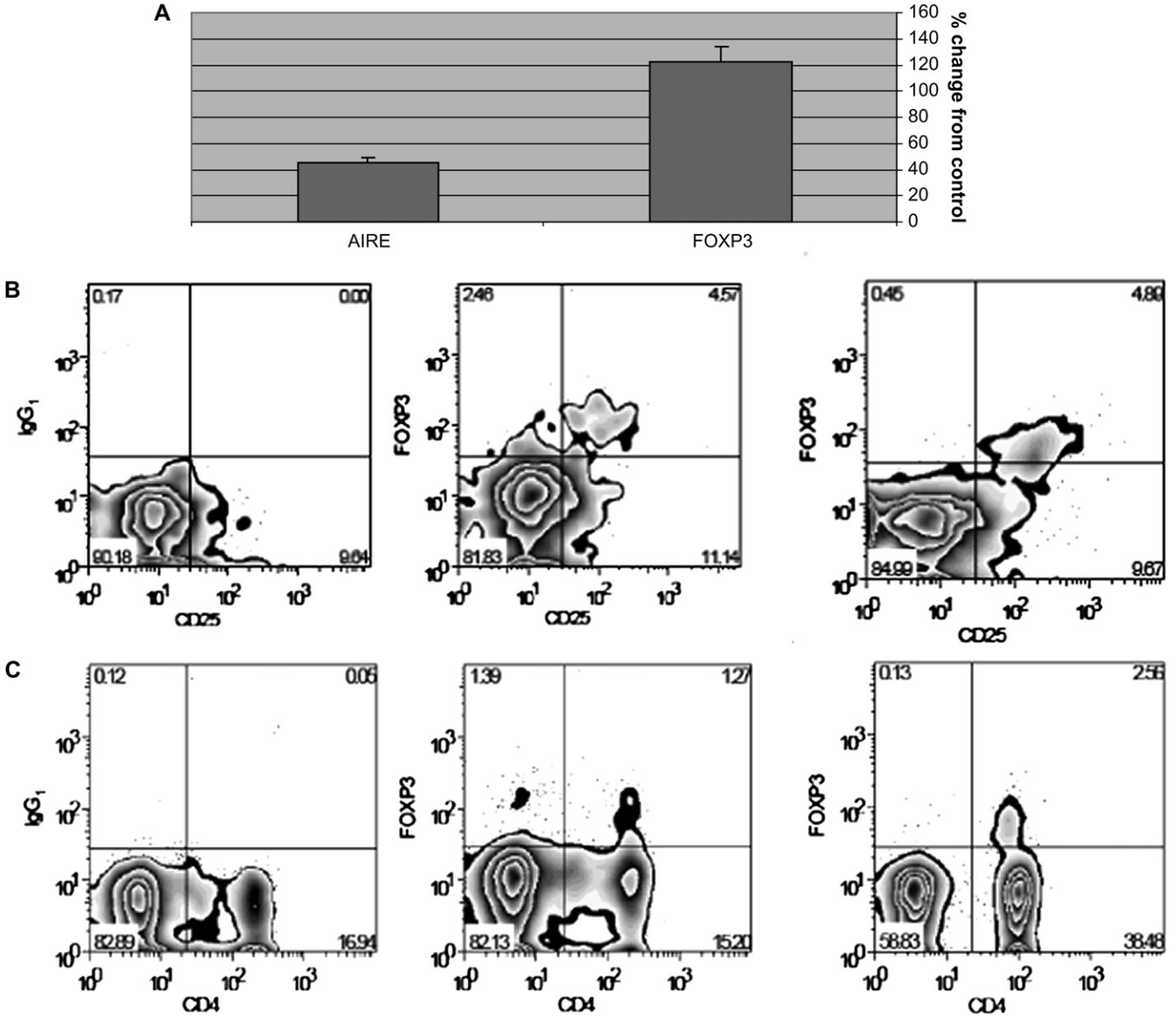


FIG 3. A, Transcript expression of AIRE and Foxp3 in PBMCs of patient relative to control normalized to 100%. **B,** CD25 and FOXP3 expression in live $CD4^+$ T-cell gate, in patient *(middle)* and control *(right)*. **C,** CD4 and FOXP3 expression in live $CD3^+$ T-cell gate in patient *(middle)* and control *(right)*. *Left,* Isotype control mAb staining.

We have shown here that NKT cell numbers as well as Foxp3 mRNA and protein expression were normal in our OS patient with OS. These findings are in contrast with the mouse model of OS, which showed a dramatic reduction of Treg cells in the thymi and peripheral tissues of a RAG2-mutated mouse.[24,25] This discrepancy may be explained by species-specific differences, which are not uncommon, or alternatively, reflects features in a mouse model that may not be duplicated in the human condition.

Transcriptional profile associated with OS features, using a broad set of genes of the human immune system, revealed a significant change in 25.5% of the tested genes. Several dysregulated genes were known to be closely involved in maintaining self-tolerance and autoimmunity and therefore could point to the mechanism underlying autoimmunity in OS. Chemokine (C-X-C motif) ligand 10, which was significantly upregulated in the patient, was also found to be markedly increased in some patients with AIRE deficiency.[26] CCR7, which is involved in organizing thymic architecture, a critical stage for AIRE function,[27] was significantly reduced in our patient. Similarly, intercellular adhesion molecule 1, which is important in positive selection along with survival of selected thymocytes,[28] a stage in which AIRE is believed to function, was also significantly downregulated in the patient. It is believed that the autoimmunity observed in OS is likely caused by a failure to express tissue-specific transcripts in the thymic medulla, secondary to reduced or absent AIRE gene expression.[14] Our findings confirm this idea and also show that early but not late TCR rearrangement occurs in the thymocytes that evade negative selection in this disease. Genes involved in the composition and function of Treg cells were found to be normal or even

TABLE II. Gene expression profile in Omenn circulating T cells

Downregulated genes		Upregulated genes	
Gene	**Fold change**	**Gene**	**Fold change**
CCR7	0.04	*IL10*	7.18
PTGS2	0.13	*CCR2*	4.68
EDN	0.14	*TNFR*	3.50
IL17	0.17	*FASL*	2.50
ECE1	0.31	*CCR5*	2.46
NFKB	0.32	*CXCL10*	2.42
ICAM1	0.35	*PRF1*	2.24
IL12	0.35	*IL2RA*	2.03
BCL2	0.38		
CD28	0.38		
CD40L	0.40		
IL1	0.40		
SELP	0.48		
CD40	0.50		
CD80	0.53		

The TLDA was used to determine the transcriptional profiles of 90 genes associated with the immune system. Twenty-three genes whose expression was significantly (>2 fold) upregulated (8 genes) or downregulated (15 genes) in the patient with OS are listed.

upregulated in the PBMCs of our patient. Moreover, the detected cytokine expression profile at the transcript level did not suggest that the function of the $FOXP3^+$ Treg cells identified in the patient's PBMCs was compromised by the cytokine milieu. The impressive upregulation of IL-10 (7.185-fold increase) in the patient's lymphocytes may have been a compensation mechanism of Treg cells to suppress the autoimmunity response induced by an abnormal central tolerance. The upregulation of CD25 can be explained by the high expression of activated T cells, a characteristic of an autoimmune response.

Signaling through costimulation ligands and receptors is a critical pathway in the regulation of T-cell activation and autoimmunity.[29] Indeed, we found downregulation of the costimulators CD40, CD40L, CD28, and CD80 transcripts, whereas the CD86 transcript was upregulated. Interestingly, the IL-17 transcript, the product of specific T_H17 cells that was critical in the induction of autoimmunity,[30] was significantly downregulated in the patient, suggesting that the T_H17 pathway is probably not a critical mechanism of autoimmunity in OS.

We have shown here for the first time that T-cell oligoclonal expansion emanates from an incomplete block before the maturation stage of negative selection, which may explain the escape of autoreactive T cells from the thymus. Indeed, we show a pattern of gene expression compatible with autoimmunity in these cells. In full agreement with low expression of AIRE in the thymus of OS,[14] we show that AIRE expression is also low in PBMCs. However, unlike the murine OS model, our patient had normal expression of Foxp3 and circulating Treg cells, suggesting that in human beings, these cells do not play a central role in developing autoimmunity in this condition.

Clinical implications: We demonstrated the role of central tolerance in the development of the autoimmune feature associated with OS. Our results are applicable to other disorders characterized by combinations of immunodeficiency and autoimmunity.

REFERENCES

1. Aleman K, Noordzij JG, de Groot R, van Dongen JJ, Hartwig NG. Reviewing Omenn syndrome. Eur J Pediatr 2001;160:718-25.
2. Corneo B, Moshous D, Güngör T, Wulffraat N, Philippet P, Le Deist FL, et al. Identical mutations in RAG1 or RAG2 genes leading to defective V(D)J recombinase activity can cause either T-B-severe combined immune deficiency or Omenn syndrome. Blood 2001;97:2772-6.
3. Ege M, Ma Y, Manfras B, Kalwak K, Lu H, Lieber MR, et al. Omenn syndrome due to ARTEMIS mutations. Blood 2005;105:4179-86.
4. Giliani S, Bonfim C, de Saint Basile G, Lanzi G, Brousse N, Koliski A, et al. Omenn syndrome in an infant with IL7RA gene mutation. J Pediatr 2006;148:272-4.
5. Roifman CM, Zhang J, Atkinson A, Grunebaum E, Mandel K. Adenosine deaminase deficiency can present with features of Omenn syndrome. J Allergy Clin Immunol 2008;121:1056-8.
6. Roifman CM, Gu Y, Cohen A. Mutations in the RNA component of RNase mitochondrial RNA processing might cause Omenn syndrome. J Allergy Clin Immunol 2006;117:897-903.
7. Grunebaum E, Bates A, Roifman CM. Omenn syndrome is associated with mutations in DNA ligase IV. J Allergy Clin Immunol 2008;122:1219-20.
8. Wada T, Yasui M, Toma T, Nakayama Y, Nishida M, Shimizu M, et al. Detection of T lymphocytes with a second-site mutation in skin lesions of atypical X-linked severe combined immunodeficiency mimicking Omenn syndrome. Blood 2008;112:1872-5.
9. Signorini S, Imberti L, Pirovano S, Villa A, Facchetti F, Ungari M, et al. Intrathymic restriction and peripheral expansion of the T-cell repertoire in Omenn syndrome. Blood 1999;94:3468-78.
10. Villa A, Notarangelo LD, Roifman CM. Omenn syndrome: inflammation in leaky severe combined immunodeficiency. J Allergy Clin Immunol 2008;122:1082-6.
11. Delves PJ, Roitt IM. The immune system: first of two parts. N Engl J Med 2000;343:37-49.
12. Suzuki E, Kobayashi Y, Kawano O, Endo K, Haneda H, Yukiue H, et al. Expression of AIRE in thymocytes and peripheral lymphocytes. Autoimmunity 2008;41:133-9.
13. Notarangelo LD, Gambineri E, Badolato R. Immunodeficiencies with autoimmune consequences. Adv Immunol 2006;89:321-70.
14. Cavadini P, Vermi W, Facchetti F, Fontana S, Nagafuchi S, Mazzolari E, et al. AIRE deficiency in thymus of 2 patients with Omenn syndrome. J Clin Invest 2005;115:728-32.
15. Bennett CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, Whitesell L, et al. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. Nat Genet 2001;27:20-1.
16. Wildin RS, Ramsdell F, Peake J, Faravelli F, Casanova JL, Buist N, et al. X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. Nat Genet 2001;27:18-20.
17. Roifman CM, Somech R, Grunebaum E. Matched unrelated bone marrow transplant for T+ combined immunodeficiency. Bone Marrow Transplant 2008;41:947-52.
18. van Dongen JJ, Langerak AW, Brüggemann M, Evans PA, Hummel M, Lavender FL, et al. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: report of the BIOMED-2 Concerted Action BMH4-CT98-3936. Leukemia 2003;17:2257-317.
19. Dik WA, Pike-Overzet K, Weerkamp F, de Ridder D, de Haas EF, Baert MR, et al. New insights on human T cell development by quantitative T cell receptor gene rearrangement studies and gene expression profiling. J Exp Med 2005;201:1715-23.
20. Douek DC, Vescio RA, Betts MR, Brenchley JM, Hill BJ, Zhang L, et al. Assessment of thymic output in adults after haematopoietic stem-cell transplantation and prediction of T-cell reconstitution. Lancet 2000;355:1875-81.
21. Kato M, Kimura H, Seki M, Shimada A, Hayashi Y, Morio T, et al. Omenn syndrome—review of several phenotypes of Omenn syndrome and RAG1/RAG2 mutations in Japan. Allergol Int 2006;55:115-9.
22. Villa A, Santagata S, Bozzi F, Imberti L, Notarangelo LD. Omenn syndrome: a disorder of Rag1 and Rag2 genes. J Clin Immunol 1999;19:87-97.
23. Peterson P, Org T, Rebane A. Transcriptional regulation by AIRE: molecular mechanisms of central tolerance. Nat Rev Immunol 2008;8:948-57.
24. Khiong K, Murakami M, Kitabayashi C, Ueda N, Sawa S, Sakamoto A, et al. Homeostatically proliferating CD4 T cells are involved in the pathogenesis of an Omenn syndrome murine model. J Clin Invest 2007;117:1270-81.
25. Wong SY, Roth DB. Murine models of Omenn syndrome. J Clin Invest 2007;117:1213-6.
26. Kisand K, Link M, Wolff AS, Meager A, Tserel L, Org T, et al. Interferon autoantibodies associated with AIRE deficiency decrease the expression of IFN-stimulated genes. Blood 2008;112:2657-66.

27. Förster R, Davalos-Misslitz AC, Rot A. CCR7 and its ligands: balancing immunity and tolerance. Nat Rev Immunol 2008;8:362-71.
28. Pollard KM, Arnush M, Hultman P, Kono DH. Costimulation requirements of induced murine systemic autoimmune disease. J Immunol 2004;173: 5880-7.
29. Vincenti F. Costimulation blockade in autoimmunity and transplantation. J Allergy Clin Immunol 2008;121:299-306.
30. Schnyder-Candrian S, Togbe D, Couillin I, Mercier I, Brombacher F, et al. Interleukin-17 is a negative regulator of established allergic asthma. J Exp Med 2006;203:2715-25.

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