

Engrafted maternal T cells in human severe combined immunodeficiency: Evidence for a T_{H2} phenotype and a potential role of apoptosis on the restriction of T-cell receptor variable β repertoire

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Engraftment of transplacentally derived maternal T lymphocytes in infants with severe combined immunodeficiency (SCID) is caused by lack of rejection of the HLA-mismatched maternal T cells by the recipient's immune system. Although the engrafted T cells express surface activation markers, they are anergic.¹ Restriction of T-cell receptor variable β (TCRV- β) segment use has been demonstrated in engrafted maternal T cells,^{2,3} but it remains to be explained how it occurs.

The high IgE levels and eosinophilia typical of Omenn's syndrome, a SCID characterized by the emergence of a limited number of autoreactive endogenous T-cell clones, has been attributed to the T_{H2} phenotype of these cells.⁴ However, little is known about the T_H phenotype of engrafted maternal T cells in other patients with SCID with elevated serum IgE levels and eosinophilia. In one such patient described here, the engrafted maternal T cells had a predominantly T_{H2} phenotype and a restricted pattern of TCRV- β gene use. In addition, evidence was obtained suggesting that the latter phenomenon might result from apoptosis.

Abbreviations used

GM-CSF:	Granulocyte-macrophage colony-stimulating factor
GVHD:	Graft-versus-host disease
SCID:	Severe combined immunodeficiency
TCRV- β :	T-cell receptor variable β
TGF- β 1:	Transforming growth factor β 1

CASE REPORT

The patient, a female and second child of unrelated parents with a negative family history for immunodeficiency disease, was hospitalized at 3 months of age because of mild erythroderma, oral candidiasis, and failure to thrive. Moderate anemia (3,370,000/mm³; hemoglobin: 9.7 gm/dl) and leukocytosis (29,000/mm³; 20% neutrophils, 29% lymphocytes, 11% monocytes, and 40% eosinophils) with hypereosinophilia and elevated serum IgE levels (109 to 481 IU/ml) were present. The patient's serum immunoglobulin level was low (IgG, 50 mg/dl; IgA and IgM undetectable). Flow cytometric analysis showed the following percentages and absolute numbers of CD3⁺ (39%, 3279/mm³), CD8⁺ (13%, 1093/mm³), CD4⁺ (35%, 2943/mm³) and CD2⁺ cells (70%, 5880/mm³). CD19⁺ cells were less than 1%. Virtually all CD3⁺ cells expressed the $\alpha\beta$ T-cell receptor and were CD4⁺ and DR⁺. No CD3/CD8 double-positive cells were detected, indicating that the CD8⁺ cells were CD3⁻. The similar percentages of CD8⁺ cells and of those coexpressing CD2/CD16 (15%) suggested an almost exclusive expression of CD8 on natural killer cells. In vitro cell cultures³ with phytohemagglutinin, phorbol myristate acetate plus ionomycin, and anti-CD3 yielded abnormally low responses (<15%

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J Allergy Clin Immunol 1998;101:131-4.

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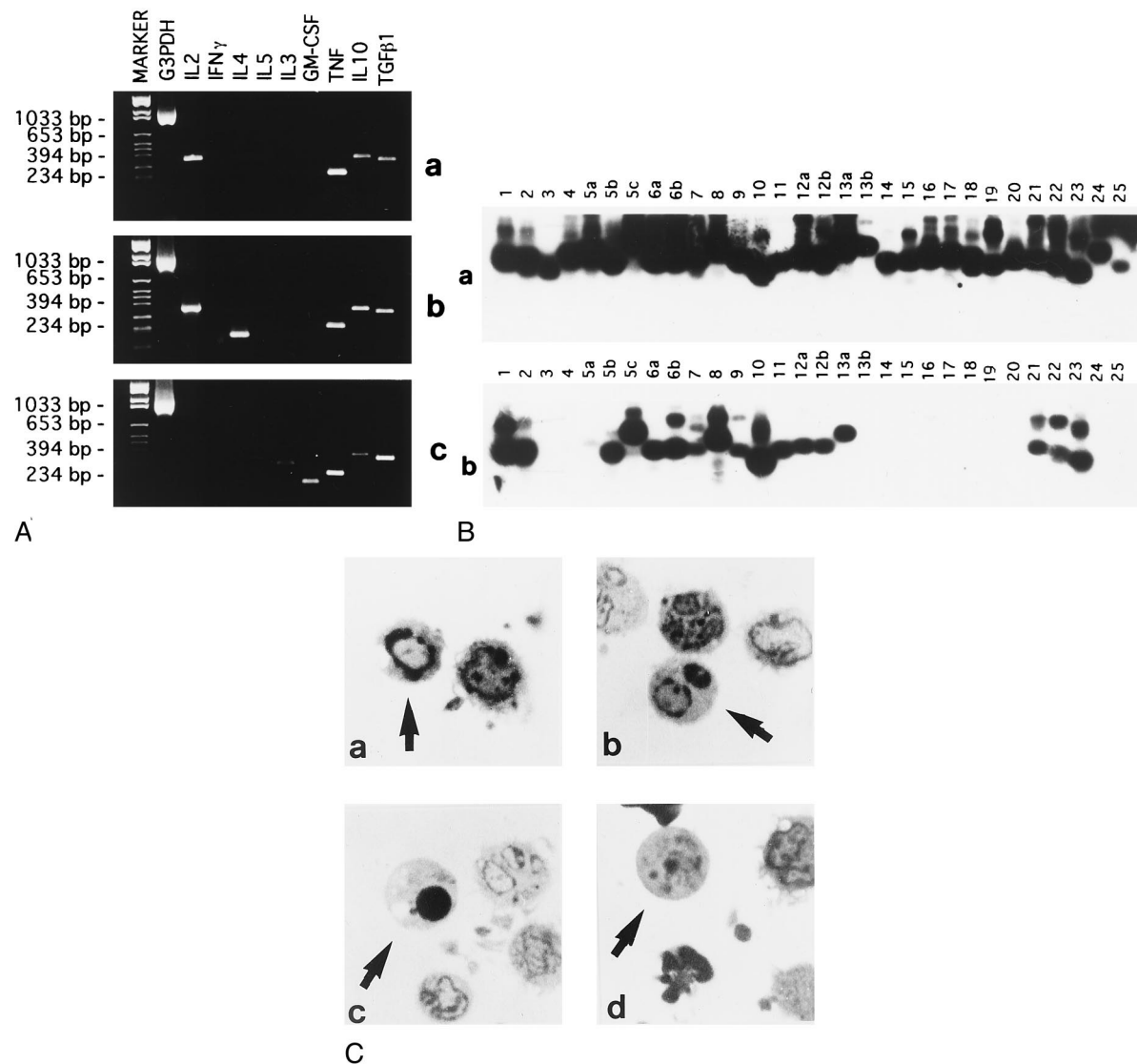


FIG. 1. **A**, mRNA cytokine expression in the mother's CD3⁺ (*a*) and in the patient's CD3⁺ (*b*) and CD3⁻ cells (*c*). **B**, Profile of TCRV- β family (numbers) expression in the mother's (*a*) and patient's (*b*) CD3⁺ cells. **C**, Early to late stages (*a* to *d*) of apoptosis observed on microscopic examination of the monocytes in the patient's CD3⁻ cell fraction (magnification $\times 700$). Enrichment was performed with magnetic microbeads coated with specific monoclonal antibodies (Dynabeads M-450). The primers and the amplification conditions for IL-3, GM-CSF, and TGF- β 1 were, respectively: F 5' AGCCACCTTTGCCTTTGCTC, R 5' TGTTGAGCCTGCGCATTTCTC, 1 minute at 94° C and 2 minutes at 70° C; F 5' ATCTCTGCACCGCCGCTCG, R 5' CCCTGCTTGTACAGCTCCAGG, 1 minute at 94° C and 2 minutes at 70° C; F 5' GCCCTGGACACCAACTATTGC, R 5' GCAGGAGCGCACGATCATGT, 1 minute at 94° C, 1 minute at 65° C, and 1 minute at 72° C.

of controls); the defective response to anti-CD3 reached normal levels after addition of human recombinant IL-2.

HLA typing by a microtoxicity assay revealed the exclusive presence of maternal haplotypes on both the patient's unseparated peripheral blood mononuclear cells and CD4⁺ enriched cells, whereas the CD8⁺ and CD4/CD8 double-negative cell subsets expressed both parents' haplotypes. In skin and lymph node biopsy specimens, CD4⁺ cells overwhelmed CD8⁺ cells, and in the skin most CD4⁺ cells were also CD45RO⁺. Features typical of graft-versus-host disease (GVHD) were evident in the

skin biopsy specimens; the corticoparacortical area was largely depleted in the lymph nodes. Adenosine deaminase and purine nucleoside phosphorylase activities were normal. The above data allowed the diagnosis of a T⁺B⁻ SCID with maternal engraftment of CD3/CD4 positive cells.

RESULTS

Cytokine gene expression⁵ showed IL-4 messenger RNA (mRNA) in the patient's CD3⁺ cells but not in her

CD3⁻ or her mother's CD3⁺ cells. It also showed IL-2 mRNA in both the patient's and her mother's CD3⁺ cells but not in the patient's CD3⁻ cells. No IL-5 or interferon- γ mRNA were detected in any of the cell fractions studied, whereas tumor necrosis factor, IL-10, and TGF- β 1 mRNA were found in all three cell subsets. Granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-3 mRNA were detected only in the patient's CD3⁻ cells (Fig. 1, A). Expression of TCRV- β genes³ revealed a restricted pattern of TCRV- β gene use in the patient's CD3⁺ cells compared with maternal CD3⁺ cells (Fig. 1, B). Flow cytometry, after 24 hours of culture in complete medium and propidium iodide staining,⁶ showed a significantly higher percentage of spontaneous apoptosis in the patient's (31.6%) than in her mother's (1.3%) CD3⁺ cells, and on microscopic examination of the patient's CD3⁻ cells, an elevated degree of apoptosis was observed among cells with the features of monocytes (Fig. 1, C).

DISCUSSION

In the infant with T⁻B⁻ SCID with hypereosinophilia and elevated serum IgE levels reported here, the finding of an engraftment of maternal CD4⁺ lymphocytes indicates a proliferative advantage, or survival, of CD4⁺ over CD8⁺ cells. Absence of CD8⁺ cells in the bloodstream cannot be attributed to their preferential tissue recruitment because CD4⁺ infiltrates were predominant in both the skin and lymph nodes. In patients with SCID, the engrafted cells are preferentially either CD4⁺ or CD8⁺,² but so far it is undetermined whether the type of SCID or the patient's and/or mother's HLA haplotype determines which subset is engrafted.

The finding of IL-4 mRNA in the engrafted, but not in the maternal, control cells and the absence of interferon- γ mRNA in both samples demonstrates the T_H2 phenotype of the engrafted cells and may explain the elevated serum IgE levels. In the absence of IL-5 transcript, the hypereosinophilia can be explained by the abundant expression of GM-CSF mRNA observed together with the faint band of IL-3 mRNA in the patient's CD3⁻ subset, as such hematopoietic growth factors released by non-T-cells can induce eosinophil differentiation and expansion *in vivo*.⁷

A restricted TCRV- β gene pattern, often present in patients with SCID, was documented by the absent expression of 13 TCRV- β genes in engrafted (CD4⁺), but not in control (CD4⁺ and CD8⁺), maternal cells. Such a finding was unlikely because of their different subset compositions. TCRV- β gene use is similar in normal CD4 and CD8 subsets. It has been demonstrated that TCRV- β subsets predominant in the maternal T cells of patients with SCID are not specific for HLA,^{2,3} leading to rejection of the hypothesis of clonotypic selection in response to host HLA. It is well known that absence of a costimulatory signal provided by antigen-presenting cells causes activated T-cell anergy and cell

death.⁸ Thus it is tempting to speculate that the high rate of apoptosis observed in this patient's monocytes may have resulted in inefficient T-cell/monocyte interaction, leading to T-cell anergy and death and consequently to restricted TCRV- β repertoire by the engrafted T cells. Whether anergic T cells may contribute to monocyte/macrophage apoptosis is not known, but antigen-presenting cell lysis by anergic T-cell clones has been reported.⁹ As in other patients with SCID,^{1,2,3} anergy of the engrafted T cells in the proband is shown by lack of proliferative response to T-cell activators, as well as by the restoration of the patient's proliferative response to anti-CD3 monoclonal antibodies in the presence of IL-2. In fact, IL-2 is known to prevent anergy that occurs when T-cell signaling is triggered in the absence of a costimulatory signal.⁸ The similar intensity of the IL-2 transcripts in the mother's resting CD3⁺ cells and in the patient's activated CD3⁺ cells suggests that an inappropriate IL-2 transcription occurs in response to activation of the patient's cells. The mild cutaneous GVHD, a further confirmation of T-cell anergy, could in part be attributed to the presence of IL-10 and TGF- β 1 transcripts, cytokines that exert an immunosuppressive effect, in the patient's CD3⁺ and CD3⁻ cells.

In conclusion, this report shows that engrafted maternal T cells in our patient with SCID had a T_H2-type pattern of cytokine production and a restricted pattern of TCRV- β gene use, which may have resulted from elimination by apoptosis of cells rendered anergic by inefficient T-cell-monocyte/macrophage interaction. Both features can explain the mild degree of GVHD observed here and in other patients with SCID with maternal engraftment.^{1,2} In this respect, animal models have shown that switch of alloreactive T cells to a type 2 cytokine phenotype has been associated with failure of acute GVHD induction after transplantation across both major histocompatibility complex class II and I barriers,¹⁰ which suggests the possibility of new approaches to prevention and treatment of acute GVHD. Patients with SCID might provide a good model for the evaluation of this issue.

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