# ABSENCE OF INTERLEUKIN 2 PRODUCTION IN A SEVERE COMBINED IMMUNODEFICIENCY DISEASE SYNDROME WITH T CELLS

By JAMES P. DISANTO,* CAROLYN A. KEEVER,‡ TRUDY N. SMALL,* GWEN L. NICHOLS,* RICHARD J. O'REILLY,‡ AND NEAL FLOMENBERG*

*From *The Effector Lymphocyte Biology and ‡Bone Marrow Transplantation Laboratories, Sloan-Kettering Institute for Cancer Research, New York, New York 10021*

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IL-2 represents the dominant growth factor for T lymphocytes, and consequently plays a central role in the development of an immune response (reviewed in reference 1). Additionally, this cytokine has been implicated in intrathymic T cell development (2, 3). Severe combined immunodeficiency disease (SCID)[1] is a rare, heterogeneous, lethal congenital disorder characterized by severe impairment of T and B cell function, normally associated with T lymphopenia (4). Although rare forms of this disease have been associated with specific enzyme or receptor deficiencies (5–7), in most cases the defects in lymphocyte development that underlie the immunodeficiency are unknown. In this report we describe an unusual SCID patient with increased T cell numbers and a defect in IL-2 production. The relevance of these findings for present models of T cell development are discussed.

## Materials and Methods

*Cellular Analysis.* PBMC were isolated by Ficoll-Hypaque gradient centrifugation. T cell proliferation assays were performed as described (8). IL-2 production was quantitated by bioassay using the IL-2-dependent murine cell line CTLL-2 (9). Immunofluorescence was performed as previously described (8), using mAbs commercially available through Becton Dickinson & Co. (Mountain View, CA) or Coulter Immunology (Hialeah, FL). Calcium mobilization was assayed using the calcium-sensitive dye FLUO-3 acetoxy-methylester (Molecular Bioprobes, Eugene, OR). PBMC were loaded with the dye as recommended by the manufacturer. Cellular fluorescence was measured at fixed intervals following crosslinking of cell surface CD3 molecules.

*Molecular Analysis.* PBMC were stimulated for 6 h and total cytoplasmic RNA was isolated following NP-40 lysis. RNA was fractionated on 1% agarose-formaldehyde gels before transfer to nylon membranes (Genescreen Plus, New England Nuclear, Boston, MA). Filters were hybridized overnight with $^{32}$P-labeled probes and washed at high stringency (0.2 × SSC, 1% SDS at 65°C). Full-length cDNA probes included human IL-2 (10), human cytotoxic serine protease B (11), human IFN-$\gamma$ (12), human granulocyte/macrophage CSF (GM-CSF) (13), human IL-2 receptor (14), and $\gamma$-actin (15). Southern blot analysis was performed on

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Address correspondence to James P. DiSanto, Ph.D., Box 41, Sloan-Kettering Institute, 1275 York Avenue, New York, NY 10021.

[1] *Abbreviation used in this paper:* SCID, severe combined immunodeficiency disease.

high molecular weight DNA isolated from an EBV-transformed B-lymphoblastoid cell line derived from the patient's B cells. Hybridization conditions were as for Northern blots.

## Case Report

The patient was the 8-lb male product of a full-term normal pregnancy. At 2 wk of age, the child developed eczema. At 2 mo of age, the child was hospitalized with bilateral otitis media and oral thrush. The patient responded to antibiotics, and was maintained on topical mycostatin. At 3.5 mo the child developed a cough with persistent mucocutaneous candidiasis. Chest x-ray revealed bronchiolitis. The patient responded to Bactrim therapy. Laboratory evaluation at this time revealed a lymphocytosis (WBC, 22.1 with 69% lymphocytes) and marked hypogammaglobulinemia (IgG 85 mg/dl; IgA <10 mg/dl; IgM 10 mg/dl). Adenosine deaminase and purine nucleoside phosphorylase levels were normal. Family history was notable for an older female sibling with recurrent bacterial and yeast infections, who died at 22 mo with autopsy-proven pneumocystis pneumonia. Parents and patient were HIV antibody negative. On initial evaluation, the patient was in the 10–25th percentile for weight, length, and head circumference. His exam was normal except for cutaneous candidiasis and eczema.

## Results

Our initial evaluation of this patient's PBMC revealed an absolute lymphocyte count of 14,400 cells/$\mu$l (normal range: 2,400–10,200 cells/$\mu$l) having normal percentages of $CD3^+$ T cells (78%) and $CD20^+$ B cells (20%). Both CD4 (helper-inducer, 62%) and CD8 (cytotoxic-suppressor, 24%) T cell subpopulations were present (CD4/CD8 ratio of 2.6). The CD4 subset contained a predominance of $CD45R^+$ cells (78%), as is normally seen early in life (16). The T cells expressed normal levels of CD2, CD3, and CD7. Cells bearing the CD25 (IL-2 receptor) and CD71 (transferrin receptor) antigens were absent (<1%). Small but detectable numbers (1–2%) of cells bearing the NK-associated markers CD16 (FcRII) and CD56 (NKH-1) were present, and the patient exhibited reduced but detectable lysis of the NK-sensitive K562 target cell line (16.6% specific lysis at 100:1 E/T ratio). Thus, in contrast to most patients with SCID, this patient presented with T and B lymphocytes of increased number, but normal distribution and phenotype.

Assays of immune function with the patient's PBMC, however, revealed absent or severely impaired responses to mitogens, common antigens, and a reduced response in MLC compared with normal controls (Table I). The combination of ionomycin and PMA could also induce weak proliferation. Addition of patient's PBMC to control MLCs failed to demonstrate any suppressor cell activity.

Strikingly, the addition of exogenous IL-2 to these in vitro assays completely restored the proliferative responses in a dose-dependent fashion (Table I). In addition, the patient's PBMC proliferated and could be propagated in vitro using immobilized mAb OKT3 and exogenously supplied IL–2. This effect could not be substituted by other lymphokines including IL-1$\beta$, IL-3, IL-4, IL-6, or GM-CSF. When PBMC were cultured for 4 d in the presence of 100 U/ml IL-2, the number of $CD3^-CD56^+$ NK cells increased to 9%, and the K562-specific lysis increased from 7.3 to 36.3% (E/T ratio of 25:1). Together, these data suggested that the patient's T cells and NK cells could respond to, but could not produce, functional IL-2 molecules. Bioassays confirmed that IL-2 was not produced following stimulation of PBMC with PHA or mAb OKT3.

Additional analyses of the T cell activation pathway were performed. The patient's cells were cultured with PHA with or without exogenous IL-2 for 3 d and the induc-

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TABLE I
*Proliferative Responses of Patient and Control PBMC*

| | Patient | Control |
|---|---|---|
| Media | 358 cpm | 533 |
| PHA | 2,222 | 78,505 |
| PWM | 1,671 | 14,213 |
| Iono/PMA | 23,429 | 70,910 |
| Media + IL-2 (U/ml) | 1,329 | 5,521 |
| PHA + IL-2 | 75,100 | 75,350 |
| Iono/PMA + IL-2 | 75,872 | 88,156 |
| *Candida albicans* | 235 | >4,000 |
| *Escherichia coli* | 250 | >2,500 |
| *Staphylococcal aureus* | 215 | >2,500 |
| MLC | 3,600 | >5,000 |

PBMC were cultured in 96-well, flat-bottomed microtiter plates at $10^5$ cells/well for 72 h and pulsed for the last 24 h with 1 $\mu$Ci/well of [$^3$H]thymidine. Mitogens were used at: PHA, 5 $\mu$g/ml; PWM, 1/100 dilution; PMA, 5 ng/ml; ionomycin, 0.3 $\mu$M. Results are the mean of three determinations with SD < 10% of the means. One of three experiments.

tion of cell surface receptors necessary for T cell proliferation, such as the CD25 and CD71 antigens, was assessed (Fig. 1). In the absence of exogenous IL-2, patient's cells expressed the CD25 and CD71 antigens, although they were slightly diminished in comparison to control cells (data not shown). When exogenous IL-2

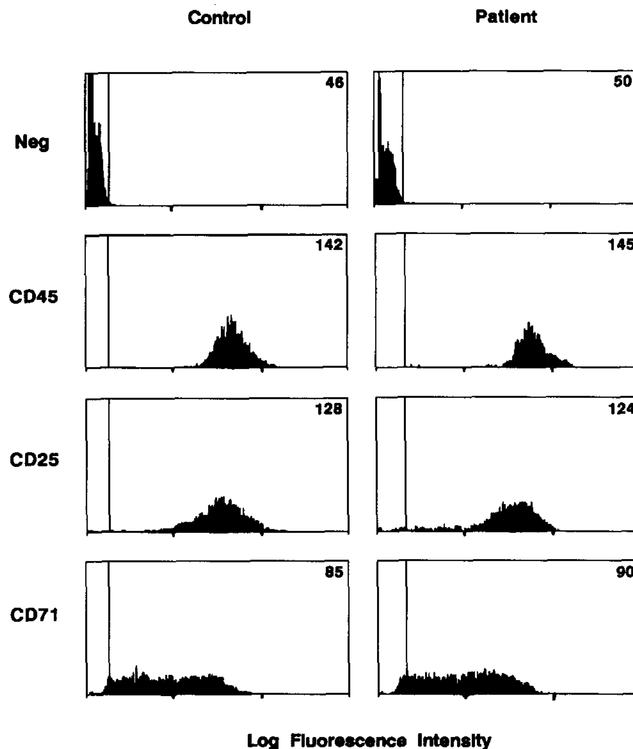


FIGURE 1. Expression of cell surface antigens following stimulation of PBMC with 5 $\mu$g/ml PHA and 25 U/ml IL-2 for 3 d. Cells were incubated with saturating amounts of negative control, CD45 (HLE), CD25 (IL2-receptor), or CD71 (OKT9) fluoresceinated mAbs and analyzed by flow cytometry. The scale of fluorescence intensity ranged from 0 to 255 over three decades of log fluorescence (85 per decade) as indicated by the three tick marks. Upper right hand corner for each histogram indicates the mean fluorescence intensity of the gated population (lymphocytes).

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was added to PHA-stimulated cultures, normal amounts of the CD25 and CD71 antigens (Fig. 1), as well as MHC class II molecules (data not shown), were expressed. The normal expression of the CD25 antigen implies that protein kinase C (PKC) activation is not defective, as these events are linked (17). In addition, the patient's PBMC demonstrated a comparable rise in intracellular calcium levels after cross-linking of surface-bound OKT3 as that seen with control PBMC (data not shown).

To determine the nature of the IL-2 production deficiency, Northern blot analysis was performed (Fig. 2). After cellular activation, control PBMC demonstrated a large increase in RNAs for IL-2, IL-2 receptor, cytotoxic serine protease-B, IFN-$\gamma$, and GM-CSF. The patient's cells produced no IL-2 transcripts under any circumstances, although both IL-2 receptor and cytotoxic serine protease B transcripts were readily induced. Induction of IFN-$\gamma$ and GM-CSF transcripts was diminished in patient's cells compared with normal controls, although a weak signal was detectable. Other lymphokine transcripts including IL-3, IL-4, and IL-6 were also present (data not shown). In vitro expanded patient's lymphocytes derived from short-term (10-d) cultures using immobilized OKT3 and exogenous IL-2 were also analyzed. As with freshly isolated PBMC, in vitro expanded patient's cells produced no IL-2 transcripts after stimulation. However, as shown in Fig. 3, IFN-$\gamma$ and GM-CSF transcripts were now readily detectable, indicating that transcription from these loci was

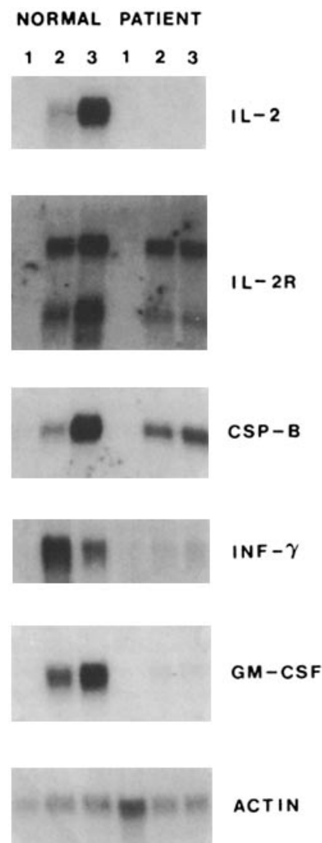


FIGURE 2. Northern blot analysis of freshly isolated PBMC following activation for 6 h with (*1*) no stimulus, (*2*) 5 ng/ml PMA and 5 $\mu$g/ml PHA, or (*3*) 5 ng/ml PMA and 0.3 $\mu$M ionomycin. 10 $\mu$g of total RNA was used for each filter. cDNA probes were labeled using $\alpha$-[$^{32}$P]dCTP. Autoradiography was for 72 h at $-70°C$ for all probes except actin which was for 24 h at $-70°C$.

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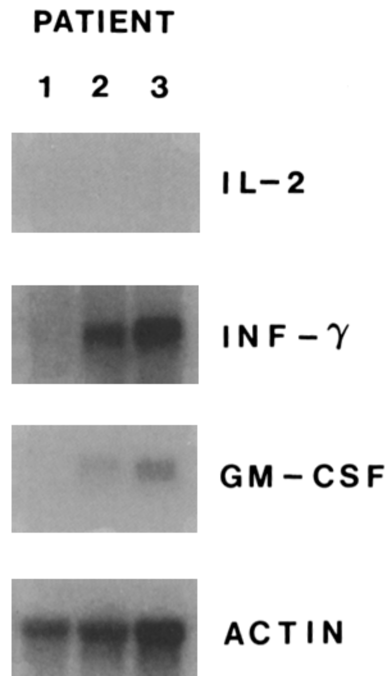


FIGURE 3. Northern blot analysis of 10-d in vitro expanded PBMC following activation for 6 h with (*1*) no stimulus, (*2*) 5 ng/ml PMA and 5 μg/ml PHA, or (*3*) 5 ng/ml PMA and 0.3 μM ionomycin. Cells were expanded using immobilized OKT3 and 25 U/ml IL-2. Autoradiography was for 24 h at −70°C.

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augmented in the presence of IL-2. Southern blot analysis using a full-length IL-2 cDNA probe revealed no differences in restriction patterns between patient's DNA and placental DNA using a variety of enzymes (Bam HI, Bgl II, Eco RI, Hind III, Pvu II, or Xba I; data not shown). All hybridizing fragments were as predicted from the known organization of the human IL-2 gene locus (18).

## Discussion

Although a variety of additional growth factors exist that augment T cell proliferation, the dominant mechanism for T cell growth is via the interaction of IL-2 with the IL-2 receptor. This autocrine pathway is critical for the generation and maintenance of functional immune responses. This patient suffers from a congenital immunodeficiency which appears to be the consequence of an inability of his circulating T cells to produce IL-2. Interestingly, of the various functional assays, the MLC response was relatively spared. This may be explained by back-production of IL-2 by the irradiated stimulator cells in response to the patient's cells, allowing the patient's cells to proliferate. The partial response to PMA and ionomycin may be due to production of other lymphokines such as IL-3, IL-4, IL-6, or GM-CSF with subsequent proliferation of responsive T and/or non-T cell populations. However, long-term in vitro growth of T cells in response to OKT3 could only be stimulated by exogenous IL-2, suggesting that the proliferative response to PMA and ionomycin might involve predominantly non-T cells.

The defect appears selective to IL-2, in that cell surface receptors and other lymphokines can be detected, although at diminished levels compared with control cells. This may be in part a consequence of absent IL-2 production, as levels increase

with the addition of exogenous IL-2. It is well established that the IL-2 receptor and IFN-$\gamma$ expression are augmented by IL-2 (19), and it appears that GM-CSF transcription may also be upregulated by IL-2. Although it remains possible that deficiencies in transmembrane signaling may be involved in the IL-2 deficiency, this seems unlikely as the patient's PBMC can mobilize intracellular calcium, and can apparently activate PKC, as evidenced by the expression of the CD25 antigen. Moreover, stimuli that bypass the cell surface (PMA and ionomycin) fail to induce IL-2 transcription.

The absence of IL-2 production may involve alterations in the IL-2 gene or in the mechanisms regulating its transcription. We found no gross structural alterations in the IL-2 gene locus, although we cannot rule out a point mutation or small deletion. Alternatively, the defect in IL-2 production may relate to an abnormality of one of the nuclear *trans*-acting proteins that bind to target sequences upstream of the IL-2 gene and regulate IL-2 transcription (20). The only T cell-specific DNA binding protein implicated in regulating IL-2 production is NFAT-1 (21). It is tempting to speculate that the patient's defect may reside in NFAT-1 or another yet unidentified transcriptional activator. Lack of such a factor might also contribute to the diminution of the other T cell-produced lymphokines (IFN-$\gamma$ and GM-CSF).

This child's genetic disorder provides important insights into T cell development which require further analysis. Specifically, this child developed and maintained a normal circulating T cell pool in the absence of detectable peripheral IL-2 production. Although IL-2 has been implicated in intrathymic T cell development (2, 3), this child's immunodeficiency disorder suggests that prethymic and intrathymic T cell development may occur in the setting of an IL-2 production defect, and reciprocally, that IL-2 deficiency does not in and of itself result in T lymphopenia. Alternatively, it may suggest that intrathymic IL-2 production may be regulated by distinct mechanisms from those that regulate IL-2 production in peripheral T cells. This child has provided important in vivo information as to the morphologic and functional consequences of peripheral IL-2 deficiency. His illness confirms the central role of IL-2 in the immune response, but suggests that data implicating IL-2 in intrathymic development must either be reconsidered, or that alternative pathways of T cell development may also exist.

## Summary

We have characterized a child with a severe combined immunodeficiency disease syndrome with increased numbers, but a normal distribution, of $CD3^+$ T cells. This patient's immunological defect appears to be attributable to a selective deficiency in T cell production of IL-2, which may reflect a subtle abnormality in the IL-2 gene locus or a defect in a regulatory factor necessary for IL-2 transcription. The increased numbers of phenotypically normal T cells in this patient suggest that alternative pathways of T cell development exist in man or that IL-2 production intra- and extrathymically is controlled via distinct regulatory mechanisms.

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