

Role of γ/δ T cells in a patient with CD4⁺CD3⁻ lymphocytosis, hypereosinophilia, and high levels of IgE

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Background: CD4⁺CD3⁻ T cells have previously been shown to play a pathogenic role in the hypereosinophilic syndrome by secreting IL-5 and IL-4.

Objectives: The goal of this study was to study the role of CD4⁺CD3⁻ and other T-cell subsets in a patient with eosinophilia, dermatitis, and a high level of IgE (100,000 IU/mL) in the serum.

Methods: We isolated PBMCs and performed flow cytometry, cell cultures, and in vitro assays of Ig, lymphokine production, and cell-mediated cytotoxicity.

Results: Flow cytometric and immunohistochemical analysis of the PBMCs revealed a major population (consisting of approximately 85% of the CD4⁺ T cells) that lacked expression of CD3 and T-cell receptors on the cell surface (CD4⁺CD3⁻ T cells), but did express CD3 peptides in the cytoplasm. Activation of the PBMCs in vitro resulted in a 100-fold greater than normal release of IL-4, whereas IFN- γ production was less than normal, suggesting a predominantly type 2 helper functional phenotype of the CD4⁺CD3⁻ T cells. Importantly, both CD4⁺CD8^{low} V δ 1⁺ T-cell receptor $\gamma\delta$ ⁺ and CD4⁺CD3⁻ T cells were cultured from the PBMCs. The former secreted IFN- γ exclusively, whereas the latter secreted both IL-4 and IFN- γ . Furthermore, only the T-cell receptor $\gamma\delta$ ⁺ lymphocytes were cytotoxic to autologous B-lymphoblastoid cells and specifically inhibited IgE production in cultures of autologous polyclonally stimulated PBMCs.

Conclusions: The results suggest that CD8^{low} V δ 1⁺ T-cell receptor $\gamma\delta$ ⁺ clones functionally counteract IgE-inducing effects of type 2 CD4⁺CD3⁻ helper cells in this patient with hypereosinophilic syndrome. (*J Allergy Clin Immunol* 1998;102:621-30.)

Key words: Hypereosinophilic syndrome, $\gamma\delta$ T cells, CD4⁺ T cells, IL-4, IFN- γ

Many studies have shown that CD4⁺ T cells can be divided into 2 functionally dichotomous classes on the basis of their production of lymphokines.¹⁻³ T_{H1} CD4⁺ T cells secrete IFN- γ and IL-2 and are chiefly involved in

Abbreviations used

cpm:	Counts per minute
FACS:	Fluorescence activated cell sorter
FM:	Final medium
HES:	Hypereosinophilic syndrome
LCL:	Lymphoblastoid B-cell line
PMA:	Phorbol 12-myristate 13-acetate
PWM:	Pokeweed mitogen
TCR:	T-cell receptor

cellular immunity, whereas T_{H2} cells produce IL-5, IL-4, IL-10, and IL-13 and induce humoral allergic responses.¹⁻³ Whereas these 2 subsets may normally regulate each other reciprocally through the activity of IFN- γ and IL-4, the hypereosinophilic syndrome (HES), consisting of hypereosinophilia and eosinophilic infiltration of the skin, lung, gastrointestinal tract, and heart, is distinguished by a pathological predominance of the T_{H2} subset.⁴⁻¹⁰ Thus in patients with "idiopathic" HES, a predominant percentage of CD4⁺ T-cell clones cultured from the peripheral blood secrete IL-5, the major eosinophilic growth factor, and several studies have identified high levels of secretion of IL-5 in HES accompanying T-cell lymphoma.⁶⁻¹⁰ IL-4, which drives the differentiation of B cells toward the synthesis and secretion of IgE, may also be overproduced and may be involved in generating the high levels of IgE and asthma seen in some patients.¹¹⁻¹³

Whereas most normal and disease-associated T_{H2} cells express the T-cell receptor (TCR) $\alpha\beta$ heterodimer and its signaling element, CD3, on the cell surface, recent reports indicate that in a subset of patients with HES, CD4⁺ T_{H2} cells lack CD3-TCR in the cell-surface membrane because of defective expression of TCR genes (CD4⁺CD3⁻ T cells).^{14,15} Whether the effects of these pathogenic lymphocytes can be influenced by other regulatory T cells (eg, T_{H1} CD4⁺ or CD8⁺ lymphocytes) has not been previously addressed. In this report we have characterized T cells in a 35-year-old woman with HES manifesting with sustained eosinophilia, severe dermatitis, and strikingly high levels of IgE in the serum. We found a dominant population of CD4⁺ T cells that lack expression of the CD3-TCR complex in the patient's peripheral blood. Moreover, her PBMCs, when activated,

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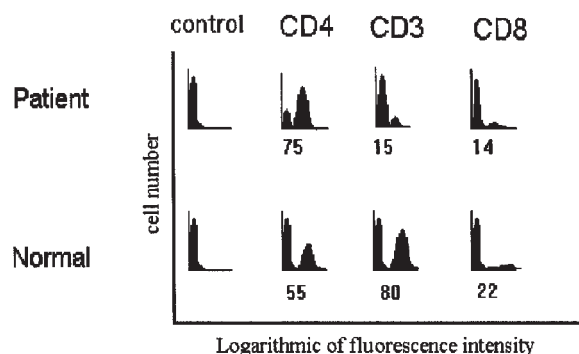


FIG 1. Representative FACS analysis of patient and normal PBMCs (0.5×10^6). Patient and control PBMCs were stained with mAb OKT4, OKT3, and OKT8 to respective surface antigens. y-axis is proportional to number of cells, x-axis to logarithmic of the fluorescence intensity (arbitrary units). Numbers indicate percentage of positive cells after subtraction of background control, consisting of staining with second antibody alone (FITC-conjugated goat anti-mouse Ig). Preliminary experiments determined that isotype control mAb gave no staining above background.

secreted abnormally high levels of IL-4 and low levels of IFN- γ , suggesting that these cells express a T_H2 pattern of lymphokine secretion. In contrast, cytotoxic CD4-CD8^{low} T-lymphocytes bearing γ/δ TCR that were cloned from the patient's peripheral blood produced IFN- γ but not IL-4 and inhibited IgE secretion in vitro.

METHODS

Patient and control subjects

The patient will be described in detail in a separate study describing 3 patients with CD4⁺CD3⁻-related HES (Reshef et al, manuscript in preparation). Briefly, she is a 35-year-old white female with severe atopic dermatitis of the hands. The white blood cell count was $11,500/\text{mm}^3$, with 35% to 40% eosinophils. Blood chemistry, chest radiography, and abdominal ultrasonogram were normal. Serum IgE were normal except for extremely elevated IgE (100,000 IU/mL, normal <100 IU/mL). Controls included normal donor blood and the patient's healthy children.

Lymphokine assays

Cells (2×10^6 cells/mL) were incubated in Falcon tubes in RPMI 1640 medium supplemented with 10% FCS (Ta'assiot Biologiot, Beit Ha'emek, Israel) and 1% penicillin-streptomycin solution (Gibco, Grand Island, NY) or in a final medium (FM) preparation consisting of Iscove's modified Dulbecco medium with the same supplements. Activation was achieved by addition of 10 ng/mL phorbol 12-myristate 13-acetate (PMA) (Sigma Chemical Co, St Louis, Mo) and 0.5 $\mu\text{g/mL}$ of the calcium ionophore A2318 (Sigma). Lymphokines in supernatants were measured by ELISA after 48 hours of culture: 96-well microtiter plates were incubated overnight at 4°C with 100 μL of a 2 $\mu\text{g/mL}$ solution of the IL-4 or IFN- γ capture antibody (Pharmingen, San Diego, Calif, or Genzyme, Cambridge, Mass, respectively) diluted in coating buffer (carbonate buffer pH 9.8) and washed 4 times with PBS, pH 7.2, plus 0.05% Tween 20. One hundred microliters of diluted standards (IL-4 or IFN- γ from Genzyme) ranging from 0 to 1000 pg/mL and of samples was added to the coated wells for 18 hours at 4°C, then

washed with buffer. One hundred microliters of the detection antibody (1 $\mu\text{g/mL}$ of biotinylated anti-human IL-4 [Pharmingen] or murine anti-human IFN- γ [Genzyme]) was added for 1 hour at room temperature. The plates were washed with PBS plus Tween-20 plus 1% BSA, and 100 μL of detection reagent (peroxidase-conjugated streptavidin or goat anti-mouse antibodies, respectively, in PBS plus 0.05% Tween-20) was added for 15 minutes at 37°C, followed by 3 washes. Substrate (tetramethylbenzidine) solution was added for 10 minutes. The reaction was stopped with 4N H_2SO_4 and the OD at 450 nm of the wells was determined by ELISA reader. Concentrations were extrapolated from the standard curve constructed from known concentrations of the lymphokines.

Determination of T-cell antigens

PBMCs were isolated from heparinized blood by Ficoll-Hypaque density centrifugation.¹⁶ To detect surface membrane antigens, 0.5 to 1×10^6 cells were spun down in 5 mL glass tubes in PBS. Resuspended pellets were stained with mAb directly conjugated with fluorochromes or with saturating concentrations of primary murine mAb at 4°C for 30 minutes and washed twice in PBS plus 0.2% sodium azide. FITC-conjugated goat anti-mouse Ig F(ab')₂ fragment of antibody (Jackson ImmunoResearch Laboratories, Inc, Westgrove, Pa) was added for 30 minutes at 4°C, and the cells were washed twice, resuspended in 0.5 mL of PBS, and analyzed on an Epics profile II Coulter Electronic fluorescence activated cell sorter (FACS) as described previously.¹⁶ For dual color staining the cells were incubated with appropriate combinations of FITC or phycoerythrin-conjugated primary mAb. Immunocytochemical studies were performed by the immunoalkaline phosphatase technique: Slides of the cytocentrifuged cells were fixed in cold acetone, then stained with mAb to the respective antigens. Rabbit anti-murine Ig and immunoalkaline phosphatase complex were added, and color was developed with naphthol ASMX phosphate plus fast red TR salt. mAbs used in these procedures were anti-CD3, anti-CD4, anti-CD8 (from Becton-Dickinson, San Jose, Calif) or from hybridomas OKT4, OKT8, and OKT3. Anti-CD5, anti-CD2, and mAb UCT3 (anti-CD3) were purchased from Dakko Co, Glostrup, Denmark. mAb TCR δ 1 (directed against constant region of TCR δ chain [C δ]) was kindly provided by M. Brenner (Harvard Medical School, Boston, Mass). BMA031 (specific for TCR $\alpha\beta$) was purchased from T-cell Sciences (Cambridge, Mass). mAb against V δ 1 (mAb A13) and V δ 2 (mAb BB3) were kindly provided by Dr L. Moretta (Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy).¹⁶ Directly conjugated mAb (anti-CD3-PE and anti-CD3-FITC, anti-CD4-FITC, and anti-CD8-PE) and their isotype-matched negative controls were purchased from Becton-Dickinson.

Cultures of T cells

Cultures were maintained in a humidified atmosphere incubator containing 7% CO_2 at 37°C; 0.5 to 1×10^6 PBMC/well were placed in 12-well tissue culture plates (Costar) in FM. Recombinant IL-2 (100 IU/mL, proleukin, Chiron, Amsterdam) was added twice weekly. Residual CD4⁺ cells were removed from growing cultures by incubating the cells at 4°C on anti-CD4-coated plates for 1 hour. Nonadherent cells were removed by pipetting and replaced in culture. Cloning of the cultured cells was performed by limiting dilution and seeding in round-bottomed 96-well microtiter plates containing irradiated "feeders" (2000 rad, autologous freshly isolated PBMC, 50,000 cells/well). These were replaced by irradiated (7000 rad) cells of an autologous lymphoblastoid B-cell line (LCL) derived by EBV transformation of B cells in the PBMCs, which were subsequently added to the wells every 10 days. An allogeneic LCL was prepared from the PBMCs of an unrelated patient.

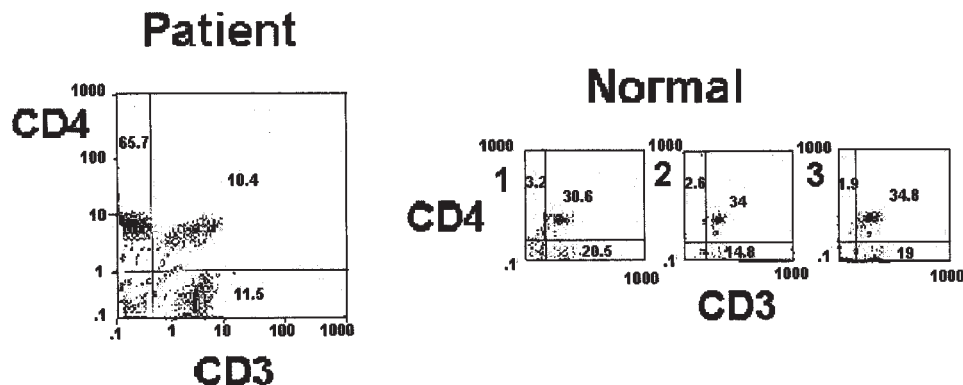


FIG 2. Detection of CD4⁺CD3⁻ T cells in patient PBMCs. Patient and normal PBMCs (from 3 of the patient's children) were stained simultaneously with anti-CD4 conjugated with PE (vertical axis) and anti-CD3 conjugated with FITC (horizontal axis). Numbers show percentage of CD4⁺CD3⁻, CD4⁺CD3⁺, and CD3⁺CD4⁻ subsets in left upper, right upper, and right lower quadrants, respectively. Staining with matching negative controls (irrelevant murine Ig) conjugated with respective dyes yielded homogeneous population of cells clustering in left lower quadrant (not shown).

TABLE I. Antigens on cell surface of patient PBMCs

Antigen	Percentage of cells per patient (range, 4 determinations)	Percentage of cells (mean, 3 normal donors)
CD2	79-82	76
CD5	80 (n = 1)	72
CD4	59-73	48
CD8	2.9-17.5	20
CD3	6.3-16.3	65
TCRαβ	7-15	60
TCRγδ	0.8-3.8	3
CD20	1-5	8

Cytotoxicity

Assays were performed as previously described.¹⁶ Target cells (10⁶) were labeled with 0.1 to 0.2 mL of Na₂⁵¹CrO₄ (Amersham, International, Buckinghamshire, UK) for 1 hour, washed 3 times of free radioactive salt, and placed (4000 cells per well) in round-bottomed 96-well microtiter plates. Effector cells were added at various effector:target ratios in FM to a volume of 200 μL/well. Plates were centrifuged (1000 rpm) for 5 minutes and placed in the humidified incubator for 4 hours. Supernatant (100 μL/well) was analyzed on a gamma counter. Percentage of specific cytotoxicity was calculated by using the mean of triplicate experiments: [experiment (counts per minute [cpm]) – spontaneous release (cpm)] × 100 / [total counts (cpm) – spontaneous release (cpm)]. Spontaneous release = cpm released in the absence of effector cells; total counts = cpm released after lysis of the targets with triton 1% solution; experiment = target + effector cells.

Ig production

Freshly isolated PBMCs (1 to 2 × 10⁶) were cultured for 8 days in plastic tubes (Elkay Products, Boston, Mass) in a total volume of 1 to 2 mL of FM containing a 1:100 dilution of pokeweed mitogen (PWM) (Sigma). Cultured lymphocytes were added as indicated in the respective experiments. Cell-free supernatants were removed and frozen at -80° C. Assays of IgG were performed by ELISA. Plates were coated with 1 μg/mL of isotype-specific capture antibody [mouse anti-human IgG (Jackson)]. One hundred microliters per well of the diluted supernatants and IgG standard ranging from

1.5 to 100 μg/mL were added. Isotype-specific alkaline phosphatase-conjugated antibody (2 μg/mL, Sigma) served as detection reagent. Phosphate substrate (Sigma) was added followed by OD determination at 405 nm and calculation of concentrations from the standard curve. IgE was measured with a modified ELISA kit from Elias (Freiburg, Germany).

RESULTS

CD4⁺CD3⁻ T cells in peripheral blood

FACS analyses (n = 4) revealed that despite normal percentages of expression of the pan-T-cell markers CD2 and CD5 (as well as of CD4 and CD8) in the patient's PBMCs, only 6% to 17% expressed the TCR-associated CD3 molecule or TCRαβ (Table I and Fig 1). Dual-color immunofluorescent staining confirmed that whereas as expected, more than 92% of the CD4⁺ T cells of the control subjects (3 of the patient's normal children) coexpressed CD3, only 14% of the patient's CD4⁺ cells coexpressed CD3 (CD4⁺CD3⁺) indicating that the vast majority (86%) are CD3 negative (CD4⁺CD3⁻) (Fig 2). Furthermore, immunocytochemical analysis of cytocentrifuged acetone-fixed PBMCs showed that consistent with the low percentage of CD3⁺ T cells revealed in the FACS analysis, only approximately 15% of the patient's PBMCs were strongly stained by anti-CD3 (Fig 3). A

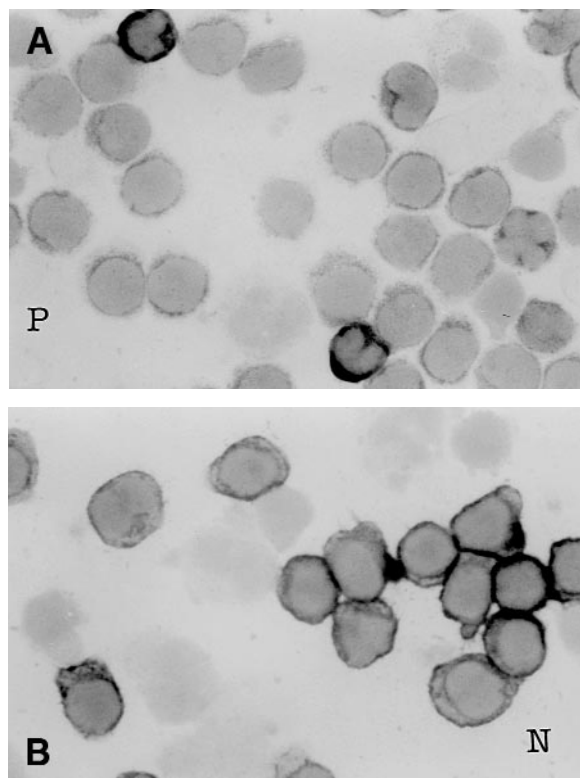


FIG 3. Intracellular CD3 in PBMCs. Cytoentrifuged, acetone-fixed PBMCs from patient (*P*) and normal (*N*) individual were stained with mAb to CD3 and photomicrographed ($\times 400$). Only a small number of patient cells (approximately 15% per high power field) are strongly stained in a manner resembling that of normal lymphocytes, but weak perinuclear intracytoplasmic staining of many of the patient's cells (approximately 80% of cells per high power field) is noted.

weak but definite staining could, however, be detected in a perinuclear distribution in the cytoplasm of approximately 80% of the PBMCs (Fig 3). Taken together, these results indicate that the large majority of the patient's peripheral blood CD4⁺ T cells express the unusual CD4⁺CD3⁻ phenotype that has previously been associated with HES.^{14,15}

Abnormally high levels of IL-4 produced by patient PBMCs

We next examined production of IL-4 and IFN- γ , lymphokines characteristically secreted by either T_{H2} or T_{H1} cells, respectively.^{1,2} Patient and control ($n = 7$) PBMCs were cultured for 48 hours either in medium alone (resting cells) or in medium plus PMA and a calcium ionophore (activated cells), and concentrations of the lymphokines in the supernatants were measured (Fig 4). IFN- γ levels were low in the supernatants of resting normal PBMCs and could not be detected in the patient's supernatants (42 ± 60.8 pg/mL [normal] vs 0 pg/mL [patient]). Activation markedly enhanced IFN- γ secretion, but the normal supernatants contained higher levels of IFN- γ than those of the patient ($22,026 \pm 3420$ pg/mL [normal] vs 7390 pg/mL [patient]). In contrast, IL-4 lev-

els were slightly higher in supernatants of the patient's resting PBMCs (165 pg/mL vs 25 ± 35.5 pg/mL in the normal control subjects). Moreover, and most strikingly, IL-4 concentration was approximately 100-fold higher in the supernatants of the patient's activated PBMCs than in the normal control subjects ($124,206$ pg/mL vs 956 ± 907 pg/mL in the normal control subjects).

T-cell lines derived from PBMCs

To establish cell lines from patient T cells, the PBMCs were cultured in medium containing recombinant IL-2 (100 U/mL) on 2 occasions. As illustrated in Fig 5, A, in experiment 1 (*ex-1*), the percentage of CD4⁺ T cells in the cultures diminished after 2 weeks, whereas that of CD3⁺ cells increased. Simultaneously, TCR $\gamma\delta$ ⁺ cells expressing the V δ 1 gene product in the TCR structure, which consisted of only 0.8% to 3.8% in the PBMCs (Table I), increased and formed the majority of the cells in the cultures after 4 weeks (Fig 5, B). In contrast, the number of cells expressing TCR $\alpha\beta$ decreased (Fig 5, B). These cultures were further purified by panning on anti-CD4-coated plastic wells, and the resulting cell line (*ex-1*) contained less than 2% CD4⁺ T cells and more than 90% TCR $\gamma\delta$ ⁺ T cells (Fig 6, A). Limiting dilutional cloning of *ex-1* yielded 4 stable CD4-CD8^{low} CD3⁺ V δ 1⁺TCR $\gamma\delta$ ⁺ clones (Table II) represented in Fig 6, C by clone B7C. In contrast to the emergence of a predominant TCR $\gamma\delta$ ⁺ population from the PBMCs in *ex-1*, a second experiment (*ex-2*) with identical culture conditions yielded a cell line (*ex-2*) expressing a uniform CD4⁺CD3⁻ phenotype, with no outgrowth of the TCR $\gamma\delta$ cells (Fig 6, B). Although limiting dilutional cloning of these cells did not yield any stable clones, the *ex-2* cells continued to express a stable CD4⁺CD3⁻ phenotype.

Functions of patient-cultured T cells

We next compared the functions of the CD4⁺CD3⁻ and the TCR $\gamma\delta$ ⁺ cells.

IL-4 and IFN- γ production. As shown in Fig 7, cells cultured in medium alone did not secrete these lymphokines spontaneously. After stimulation with PMA and calcium ionophore only, the CD4⁺CD3⁻ *ex-2* cells (Fig 6, B) secreted a substantial amount of IL-4, whereas activated CD4-CD8^{low} TCR $\gamma\delta$ ⁺ clones A4B, B7C, and AB12 did not secrete measurable amounts of this lymphokine. However, activated A4B and AB12 cells did secrete high levels of IFN- γ , and clone B7C and *ex-2* cells secreted low and intermediate levels of this cytokine, respectively.

Cytotoxicity. As shown in Fig 8, A, $\gamma\delta$ clones B7C, A6A, and AB12 were all cytotoxic to K562 cells. In addition, at a 25:1 killer:target ratio, clones A6A and B7C were weakly but significantly cytotoxic to an autologous LCL (Fig 8, B). Moreover, clone AB12 exhibited high levels of killing against the autologous LCL at both low and high killer:target ratios (Fig 8, B). In contrast, killing of allogeneic LCL by all 3 $\gamma\delta$ clones was weak or absent. The CD4⁺CD3⁻ *ex-2* cells were devoid of any cytotoxic activity against K562 or the LCL (Fig 8, A and B).

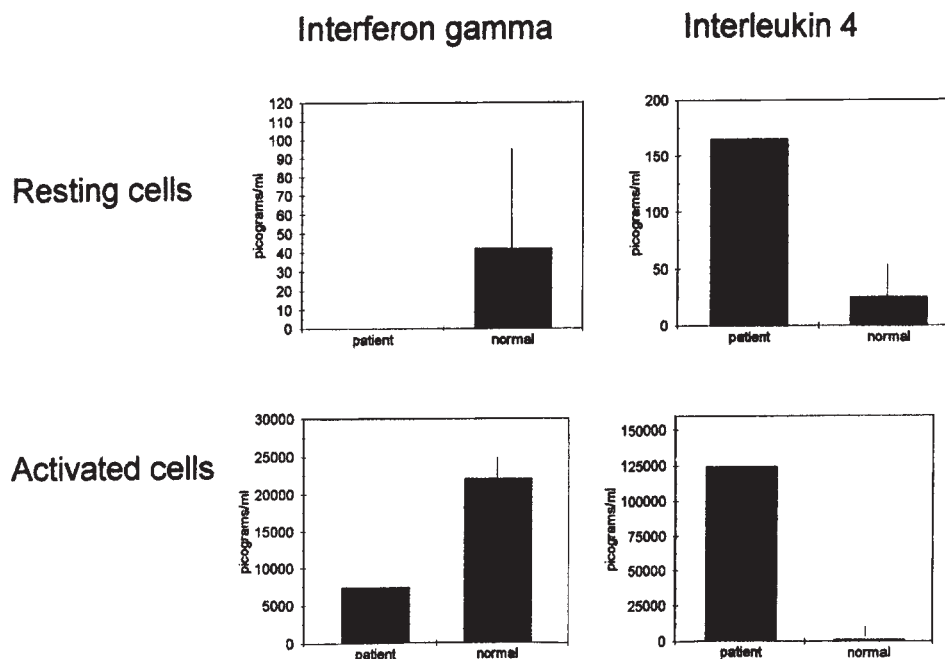


FIG 4. Cytokine production by PBMCs. IFN- γ and IL-4 (pg/mL) measured by ELISA in supernatant of 2×10^6 patient and normal control ($n = 7$) fresh PBMCs cultured for 48 hours in 2 mL medium alone (resting) or medium containing 10 ng/mL PMA and 0.5 μ g/mL of A2318 calcium ionophore (Sigma) (activated). Bars represent mean $\pm$ 1 SD of measured concentrations.

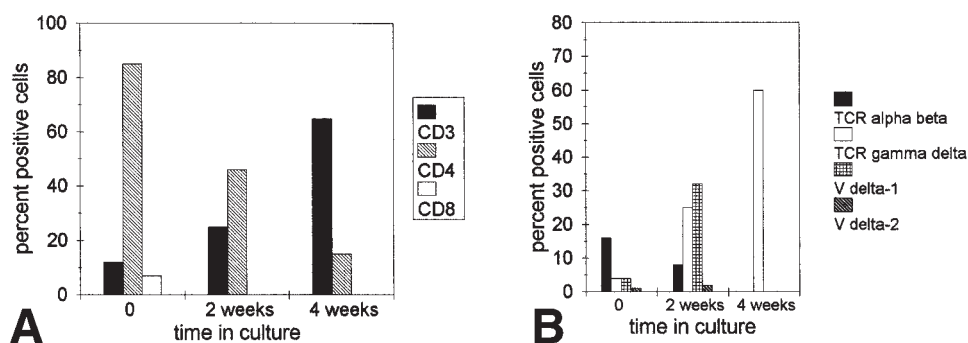


FIG 5. T-cell subsets in cultures of patient PBMCs in experiment 1. T cells were removed from wells containing patient PBMCs after culture in medium plus IL-2 for time periods indicated on abscissa. Percentage of cells expressing each indicated surface antigen was recorded after FACS analysis of cells labeled with mAbs OKT4, OKT3, and OKT8 to corresponding antigens indicated in **A** and with mAbs BMA031 (anti-TCR $\alpha\beta$), TCR δ 1 (anti-TCR $\gamma\delta$), A13 (anti-V δ 1), and BB3 (anti-V δ 2) to respective antigens indicated in **B**. Four-week culture was stained only with TCR δ 1 and BMA031.

Ig production. To determine the effect of the CD4⁺CD3⁻ and the TCR $\gamma\delta$ ⁺ T cells on the secretion of IgE by autologous B cells (consisting 1% to 5% of the PBMCs, Table I), freshly isolated PBMCs (2×10^6) of the patient and of 3 normal individuals were triggered with PWM, and concentrations of IgE were measured in the medium after 8 days of culture. Although IgE was nondetectable in the supernatants of the normal individuals (not shown), as can be seen in Fig 9, A, 1.15 ± 0.8 IU/mL of IgE were detected in the supernatant of the patient's PWM-activated PBMCs, and adding graded numbers of cells from the CD4⁺CD3⁻ ex-2 culture (Fig 6, B) to the PBMCs enhanced the secretion of IgE non-

significantly. In contrast, adding TCR $\gamma\delta$ ⁺ ex-1 T cells (Fig 6, A) significantly inhibited IgE secretion in a dose-dependent manner. In a second experiment, cells of $\gamma\delta$ clone A6A (Table II) were added to the PWM-stimulated PBMC, and IgG as well as IgE were measured in the supernatants. As shown in Fig 9, B, IgE was decreased, whereas IgG was increased in the presence of the $\gamma\delta$ clone. These results demonstrate that the suppression induced by the $\gamma\delta$ T cells is specific for the IgE isotype.

DISCUSSION

This analysis of T cells in the peripheral blood of a patient with severe dermatitis, hypereosinophilia, and

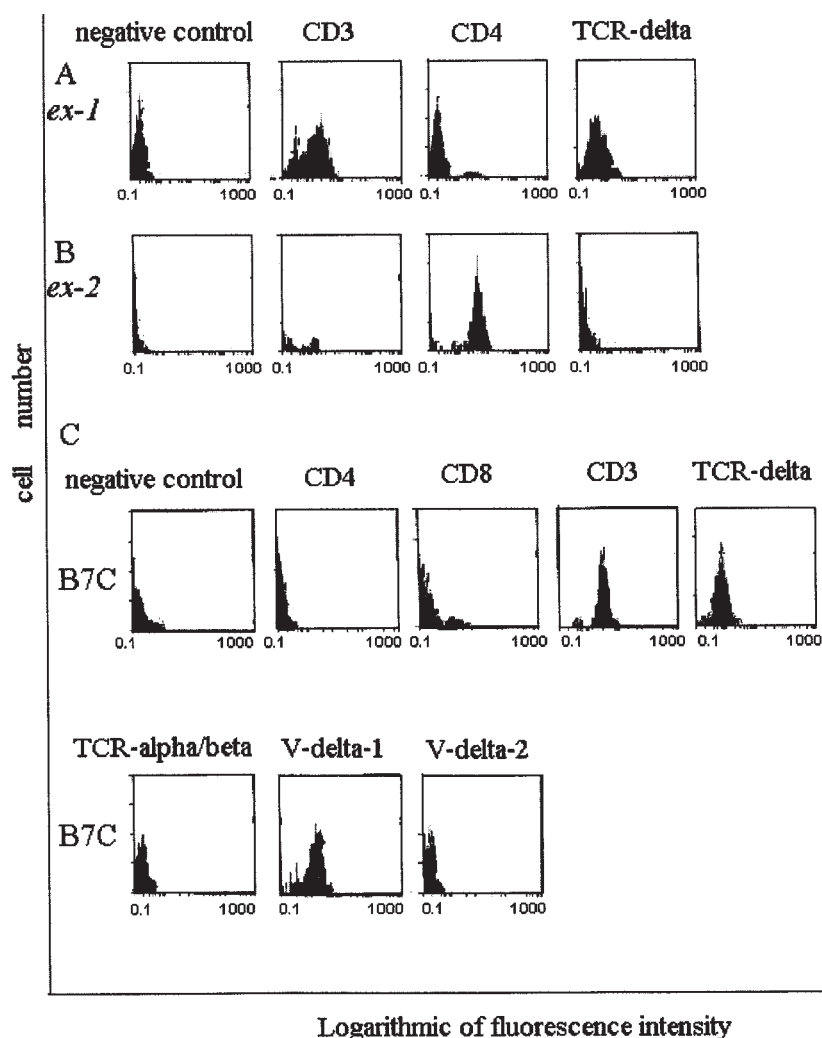


FIG 6. Antigens expressed on selected cell lines from patient's cultured T cells. Representative FACS analysis of **A**, CD4⁺CD3⁺TCR $\gamma\delta$ ⁺ T cells derived in experiment 1; **B**, CD4⁺CD3⁻ cells, derived in experiment 2; **C** representative $\gamma\delta$ clone (clone B7C, see Table II) derived from experiment-1 culture.

very high IgE in the serum revealed 4 major findings. First, the predominant T-cell population in the PBMCs expresses a CD4⁺CD3⁻TCR⁻ phenotype. Second, in vitro activation induces abnormally high secretion of IL-4 by the patient's lymphocytes. Third, CD4⁺CD8^{low} V δ 1⁺TCR $\gamma\delta$ ⁺ cells and CD4⁺CD3⁻ T cells could be expanded from the patient's peripheral blood. Finally, the former cells secrete IFN- γ exclusively of IL-4 and inhibit IgE secretion by autologous B cells.

Our results support the previous data indicating a linkage between lymphoproliferations of cells bearing the CD4⁺CD3⁻ phenotype and HES.^{14,15,17} CD4⁺CD3⁻ lymphocytes can be detected in normal thymus, where they differentiate from CD3⁺CD4⁻CD8⁻ thymic precursors and subsequently mature into CD3⁺TCR⁺CD4⁺ or CD3⁺TCR⁺CD8⁺ T cells.¹⁸ Although such cells are

rarely found in normal adult peripheral blood (Fig 2), in some patients with HES or cutaneous T-cell leukemia, individual CD4⁺CD3⁻ cells with clonal rearrangements of TCR β gene or TCR γ genes expand to comprise a major proportion of the peripheral blood lymphocytes.^{14,15,19} Alternatively, as briefly reported by Schandene et al¹⁷ and confirmed in the case described in this report (data not shown), the CD4⁺CD3⁻ T cells in HES proliferate polyclonally, as inferred by the inability to detect a monoclonal rearrangement of TCR genes in the peripheral blood by Southern blot analysis. The finding of CD3 components in the cytoplasm of the cells in our patient (Fig 3) and in previously described cases, coupled with the absence of mRNA of TCR genes in the cytoplasm, suggests that the lack of expression of TCR-CD3 complexes on the surface membrane of CD4⁺CD3⁻

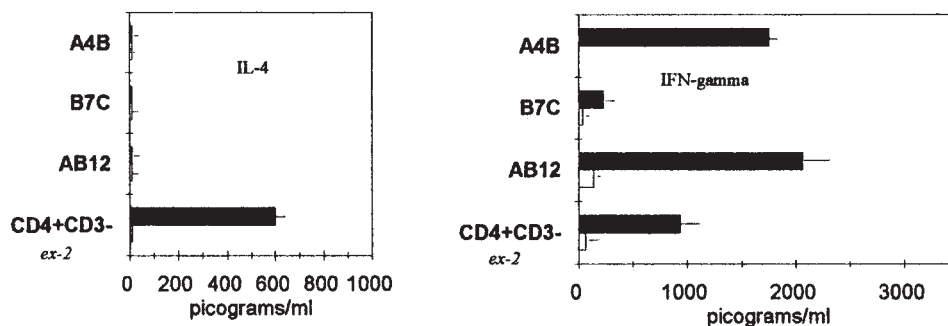


FIG 7. IL-4 and IFN- γ production by cultured T cells. A4B, B7C, and AB12 $\gamma\delta$ clones and CD4 $^{+}$ CD3 $^{-}$ ex-2 cells were removed from 24-well tissue culture plates in which they were maintained in IL-2 containing medium and washed; 0.5×10^6 cells were placed in plastic tubes in 0.5 mL of fresh FM alone (white bars) or in fresh FM containing 10 ng/mL PMA and 0.5 μ g/mL of A2318 calcium ionophore (Sigma) (black bars). Concentration of cytokines in cell-free supernatants was measured after 48 hours of culture. Bars represent mean $\pm$ 1 SD of triplicate cultures.

cells is the result of abnormal expression of TCR genes rather than of genes encoding CD3 peptides.^{15,19}

In accordance with the previously suggested concept that CD4 $^{+}$ CD3 $^{-}$ T cells in HES are T_{H2} lymphocytes that secrete IL-5 alone or IL-5 as well as IL-4, we found an approximately 100-fold above normal production of IL-4 by the patient's activated PBMCs, of which approximately 67% are CD4 $^{+}$ CD3 $^{-}$ T cells (Fig 2, A and Fig 4).^{14,15,17} Coupled with the low level of production of IFN- γ (Fig 4), it is reasonable to assume that an imbalance in the production of lymphokines, including high levels of IL-4 and perhaps other factors (eg, IL-13) contributed by the CD4 $^{+}$ CD3 $^{-}$ cells, may play a role in inducing and maintaining the high levels of IgE in the patient's serum (Fig 4).^{2,3,12} A preliminary experiment in our laboratory has in fact demonstrated that removal of the patient's CD4 $^{+}$ T cells, the vast majority of which are CD3 $^{-}$, but not of her CD3 $^{+}$ cells (including TCR $\alpha\beta^{+}$ and TCR $\gamma\delta^{+}$ cells) before triggering with PWM, reduced IgE levels measurable in the supernatants by 67% (data not shown). In this context it is of interest that IL-4 and IFN- γ were found in more or less equivalent amounts in supernatants of activated cells from cultures containing more than 90% CD4 $^{+}$ CD3 $^{-}$ T cells (line ex-2, Fig 6, B, and Fig 7). This suggests that the pattern of lymphokines produced by CD4 $^{+}$ CD3 $^{-}$ T cells is altered during in vitro culture, possibly by factors in the culture medium (including IL-2). Together with the report of Schandene et al, indicating that IFN- α downregulates IL-5 production by CD4 $^{+}$ CD3 $^{-}$ T cells in vivo and in vitro, the results suggest that both the IL-5 and IL-4 lymphokines secreted by these T_{H2} cells are susceptible to manipulation by exogenously administered lymphokines.^{14,17}

Although CD4 $^{+}$ TCR $\alpha\beta^{+}$ T_{H2} lymphocytes are the major subset of T cells responsible for the activation of B cells toward the production of IgE, recent studies suggest a role for $\gamma\delta$ T cells in controlling Ig production.²⁰⁻²⁵ This subset of T cells comprises approximately 4% of the T lymphocytes in the peripheral blood in normal individuals.²⁴ The majority of $\gamma\delta$ T cells in the peripheral blood in normal individuals use the V γ 9(2) gene segment in

TABLE II. $\gamma\delta$ T-cell clones derived from patient PBMCs

	TCR $\gamma\delta$	V δ 1	V δ 2	CD8	CD4
A4B	+	+	—	low	—
AB12	+	+	—	low	—
A6A	+	+	—	low	—
B7C	+	+	—	low	—

Antigens expressed by clones indicated in far left column. For representative FACS analysis see Fig 6, C.

conjunction with V δ 2 in the γ and δ chains of the TCR, respectively, and recognize nonpeptidic microbial derived antigens through V γ 9(2).²⁶ Although most $\gamma\delta$ clones do not express CD4 or CD8, CD4 $^{+}$ TCR $\gamma\delta$ clones have been isolated and were shown to secrete IL-4 and help B cells to secrete IgE.^{21,24} Furthermore, CD4 $^{+}$ $\gamma\delta$ T cells are increased in bronchial tissue in allergic asthma, and $\gamma\delta$ T cells are decreased in the peripheral blood of patients with allergic manifestations and asthma.^{22,23} On the other hand, $\gamma\delta$ T cells expressing the V δ 1 gene segment are normally predominant among intraepithelial lymphocytes in mucosae and are less frequent than V δ 2 $^{+}$ $\gamma\delta$ T cells in peripheral blood of normal individuals.²⁷ V δ 1 $^{+}$ $\gamma\delta$ T cells usually do not express V γ 9(2), are found in the postnatal thymus, and may form the predominant $\gamma\delta$ population in the peripheral blood in chronic inflammatory diseases.²⁷⁻³⁰ Recent studies have ascribed this preferential expansion to the fact that the V δ 1 $^{+}$ $\gamma\delta$ T cell subset specifically becomes activated by B cells activated in the patients.³¹ This suggests that interactions with autologous B cells (possibly IgE secretors activated by CD4 $^{+}$ CD3 $^{-}$ cells) may have led to the activation of V δ 1 $^{+}$ $\gamma\delta$ T cells in the patient and therefore to their preferential expansion in vitro under the influence of IL-2, as observed in ex-1 (Fig 5).

The $\gamma\delta$ cells that were derived from the patient's peripheral blood expressed low levels of CD8 (Table II and Fig 6, C) and secreted IFN- γ (Fig 7). Interestingly, in a murine model, it has been demonstrated that administration of aerosolized antigen activates IFN- γ secreting

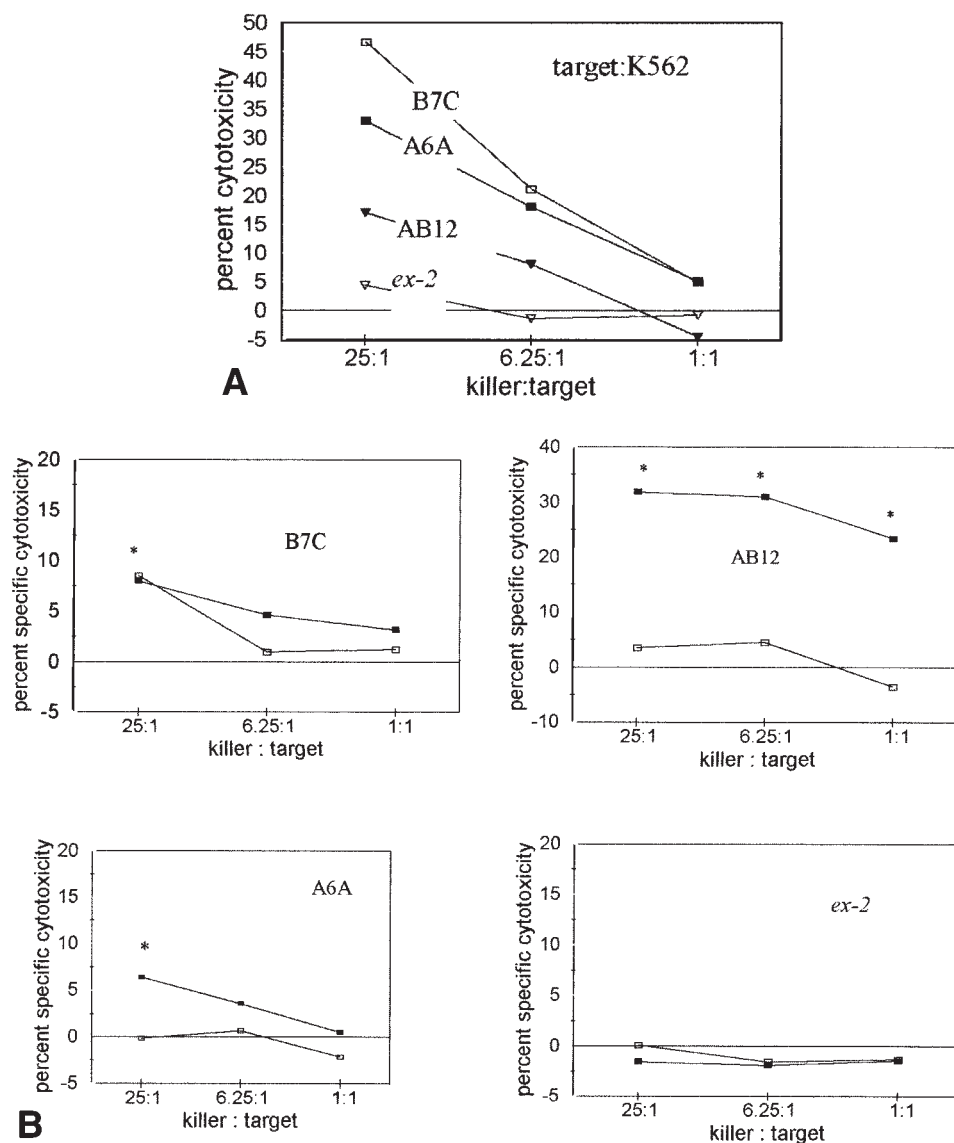


FIG 8. Cytotoxicity assays. **A**, Killing of ^{51}Cr -labeled cells of K562 line. Specific cytotoxicity in 4-hour killing assays by B7C, A6A, and AB12 $\gamma\delta$ clones and by experiment-2 line as indicated in diagrams. Results are means of triplicate experiments at indicated killer/target ratios. **B**, Specific cytotoxicity of $\gamma\delta$ T-cell clones and of experiment-2 cells as indicated within each diagram against autologous (black squares) and allogeneic (white squares) LCL. *Results in which mean experimental ^{51}Cr release is greater than 3 SD above mean of spontaneous release.³²

$\text{CD8}^+\text{TCR}\gamma\delta^+$ cells that inhibit the IgE secretion induced by $\text{CD4}^+\text{TCR}\alpha\beta^+\text{T}_{\text{H}2}$ lymphocytes.³² That the patient-derived $\gamma\delta$ cells can function as IgE suppressors in a manner analogous to $\text{CD8}^+\gamma\delta$ cells described in this murine model was demonstrated by the experiments in which we showed that the addition of patient $\gamma\delta$ cells to autologous PWM-activated PBMCs, containing large amounts of $\text{CD4}^+\text{CD3}^-$ IL-4-secreting cells, dramatically decreased the concentration of IgE in the culture supernatant (Fig 9, A). It should be noted however, that in these experiments, $\gamma\delta$ cells were added in excess of their relative number in the PBMCs, suggesting that such inhibitory effects in vivo would only be expected to

occur in microenvironments where similar ratios of $\gamma\delta$ to $\text{CD4}^+\text{CD3}^-$ cells might exist. Studies by Hyjek et al,³² showing that interactions between $\text{V}\delta 1^+\text{TCR}\gamma\delta^+$ cells and B cells induce IFN- γ secretion by the former, together with the results showing that the $\text{V}\delta 1^+\text{TCR}\gamma\delta^+$ cells from the patient produce IFN- γ when they are activated, suggest that this cytokine may be involved in the suppression of the IgE response and the concomitant augmentation of IgG secretion (Fig 9, B), although other cytokines such as IL-12 may also be involved.³ Given the cytotoxic function of the $\text{V}\delta 1^+\text{TCR}\gamma\delta^+$ clones (Fig 8), it is tempting to speculate that interactions between IgE expressed in the cell membrane of B cells and high-affin-

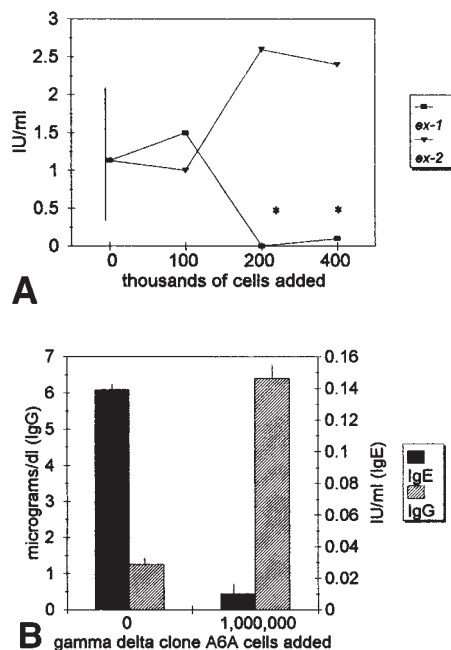


FIG 9. Ig production effect of $\gamma\delta$ T cells. **A**, Concentrations of IgE in cell-free supernatants of 4×10^6 freshly isolated patient PBMCs cultured in 2 mL FM plus PWM (1:100) for 8 days alone or were cocultured with increasing numbers of the CD4⁺CD3⁻ experiment-2 cells or CD3⁺TCR $\gamma\delta$ ⁺ experiment-1 T cells depicted in Fig 6, A and B. Results are given as mean $\pm$ 1 SD of triplicate (for 0 cells added) or mean of duplicate experiments. *Statistically significant difference ($P < .05$, z test) compared with mean in the absence of added cells. **B**, PBMCs (1×10^6 cells per culture in 1 mL of FM plus 1:100 dilution of PWM) were cultured for 8 days alone or with 1×10^6 cells of A6A $\gamma\delta$ 1⁺TCR $\gamma\delta$ ⁺ clone (Table II). IgE (IU/mL, y-axis on right) and IgG (mg/mL, y-axis on left) were measured in cell-free supernatant after 8 days of culture. Bars indicate mean $\pm$ 1 SD of concentrations of Ig.

ity Fc ϵ receptors that consist part of the TCR-CD3 complex in some $\gamma\delta$ clones could also contribute to the suppression of IgE by directing the clones to kill IgE-producing B cells.³³ The ability of the CD8⁺ $\gamma\delta$ 1⁺ TCR $\gamma\delta$ ⁺ clones to kill autologous LCL also suggests that the patient's CD8^{low} $\gamma\delta$ 1⁺ TCR $\gamma\delta$ ⁺ clones play an important role in immune surveillance against virally transformed B cells in this immunocompromised patient (Fig 8). Previous studies have shown, both in normal individuals and in patients with AIDS, that the killing of LCL by CD8⁺ $\gamma\delta$ 1⁺ TCR $\gamma\delta$ ⁺ clones occurs through recognition of idiotypic components of Ig in the B-cell membrane, of the CD48 surface molecule, or of antigens preferentially expressed on the activated B cells.^{31,34-37}

In summary, our results suggest that interactions among $\gamma\delta$ 1⁺ TCR $\gamma\delta$ T cells, CD4⁺CD3⁻ T cells, and B cells may have important implications for IgE regulation and immune protection in patients with HES-associated CD4⁺CD3⁻ lymphoproliferative disorders. Most importantly, despite the low number of $\gamma\delta$ 1⁺ TCR $\gamma\delta$ T cells in the peripheral blood, such cells can be expanded in vitro and have been shown in this report to have functional

properties consistent with a potential to downregulate the detrimental effects of T_{H2} cells in these patients. Thus although at present there are no data to indicate that the $\gamma\delta$ cells are influencing disease progression in the patient, studies of the role of these IFN- γ producing regulatory $\gamma\delta$ 1⁺ TCR $\gamma\delta$ ⁺ T-lymphocytes appear to be justified for further understanding and, perhaps, controlling HES.

REFERENCES

- Mosmann TR, Coffman RL. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol* 1989;7:145-73.
- Romagnani S. Th1 and Th2 in human diseases. *Clin Immunol Immunopathol* 1996;80:225-35.
- Borish L, Rosenwasser L. Th1/Th2 lymphocytes: doubt some more. *J Allergy Clin Immunol* 1997;99:160-4.
- Maggi E, Parronchi P, Manetti R, Simonelli C, Picinni MP, Rugiu FS, et al. Reciprocal regulatory effects of IFN gamma and IL-4 on the in vitro development of human Th1 and Th2 clones. *J Immunol* 1992;148:2142-7.
- Weller PF, Bubley GJ. The idiopathic hypereosinophilic syndrome. *Blood* 1994;83:2759-79.
- Raghavachar A, Fleisher S, Frickhofen N, Heimpel H, Fleischer B. T lymphocyte control of human eosinophilic granulopoiesis: clonal analysis in an idiopathic hypereosinophilic syndrome. *J Immunol* 1987;139:3753-8.
- Screzenheimer H, Home SD, Tewald F, Fleischer B, Raghavachar A. Interleukin 5 is the predominant eosinophilopoietin produced by cloned T lymphocytes in hypereosinophilic syndrome. *Exp Hematol* 1993;21:358-65.
- Murata K, Yamada Y, Kamihara S, Atogami S, Tsukasaki K, Momita S, et al. Frequency of eosinophilia in adult T cell leukemia/lymphoma. *Cancer* 1992;69:966-72.
- Kim CJ, Park SH, Chi JG. Idiopathic hypereosinophilic syndrome terminating as disseminated T-cell lymphoma. 1991;67:1064-9.
- Samoszuk M, Ramzi E, Cooper DL. Interleukin 5 mRNA in three T cell lymphomas with eosinophilia. *Am J Hematol* 1993;42:402-4.
- Borish L, Dishuck J, Cox L, Mascali JJ, Williams J, Rosenwasser LJ. Sezary syndrome with elevated serum IgE and hypereosinophilia: role of dysregulated cytokine production. *J Allergy Clin Immunol* 1993;92:123-31.
- Paul WE. Interleukin 4: signalling mechanisms and control of T cell differentiation. *Ciba Found Symp* 1997;204:208-16.
- Corry DB, Folkesson HG, Warnock ML, Erle DJ, Matthay MA, Wienerkronish JP, et al. Interleukin 4, but not interleukin 5 or eosinophils, is required in a murine model of acute airway hyperreactivity. *J Exp Med* 1996;183:109-17.
- Cogan E, Schandene L, Crusiaux P, Velu T, Goldman M. Brief report: clonal proliferation of type 2 helper T cells in a man with the hypereosinophilic syndrome. *N Engl J Med* 1994;330:535-8.
- Brugnoni D, Airo P, Rossi G, Bettinardi A, Simon HU, Garza L, et al. A case of hypereosinophilic syndrome is associated with the expansion of a CD3-CD4⁺ T-cell population able to secrete large amounts of interleukin-5. *Blood* 1996;87:1416-22.
- Bank I, Book M, Cohen L, Kneller A, Rosenthal E, Pras M, et al. Expansion of a unique subpopulation of cytotoxic T cells that expresses a C α γ 1 T cell receptor gene in a patient with severe persistent neutropenia. *Blood* 1992;81:3157-63.
- Schandene L, Del Prete GFF, Cogan E, Stordeur P, Crusiaux A, Kennes B, et al. Recombinant interferon alpha selectively inhibits the production of interleukin-5 by human CD4⁺ T cells. *J Clin Invest* 1996;97:309-15.
- Kraft DL, Weissman IL, Waller E. Differentiation of CD3-4⁺ human fetal thymocytes in vivo: characterization of a CD3-4⁺ intermediate. *J Exp Med* 1993;178:265-77.
- Fischer MB, Hauber I, Fodinger M, Wolf HM, Thon V, Donath P, et al. Defective TCR surface expression associated with impaired TCR beta-chain assembly in a patient with cutaneous T-cell lymphoma. *J Invest Dermatol* 1995;104:537-40.
- Wen L, Pao W, Wong FS, Peng Q, Craft J, Zheng B, et al. Germinal center formation, immunoglobulin class switching, and autoantibody production driven by "non alpha/beta" T cells. *J Exp Med* 1996;183:2271-82.

21. Gascan H, Aversa GG, Gauchat J-F, vanVlasseiaer P, Roncarolo MG, Yssel H, et al. Membranes of activated CD4⁺ T cells expressing T cell receptor (TcR) $\alpha\beta$ or TcR $\gamma\delta$ induce IgE synthesis by human B cells in the presence of IL-4. *Eur J Immunol* 1992;22:1133-41.
22. Spinoza F, Elisabetta A, Bistoni O, Forenza N, Monaco A, Bassotti G, et al. Increased allergen specific steroid sensitive gamma delta T cells in bronchoalveolar lavage fluid from patients with asthma. *Ann Intern Med* 1996;124:223-7.
23. Chen KS, Miller KH, Hengehold D. Diminution of T cells with gamma delta receptor in the peripheral blood of allergic asthmatic individuals. *Clin Exp Allergy* 1994;26:295-302.
24. Kaufmann SHE. $\gamma\delta$ and other unconventional T lymphocytes: what do they see and what do they do? *Proc Natl Acad Sci U S A* 1996;93:2272-9.
25. Rizzo LV, DeKruyff RH, Umetsu DT, Caspi RR. Regulation of the interaction between Th1 and Th2 T cell clones to provide help for antibody production in vivo. *Eur J Immunol* 1995;25:708-16.
26. Tanaka Y, Morita CT, Tanaka Y, Nieves E, Brenner MB, Bloom BR, et al. Natural and synthetic non peptide antigens recognized by human $\gamma\delta$ T cells. *Nature (Lond)* 1995;375:155-8.
27. Soderstrom K, Bucht A, Halapi E, Lundqvist C, Gronberg A, Nilsson E, et al. High expression of V γ 8 is a shared feature of human $\gamma\delta$ T cells in the epithelium of the gut and in the inflamed synovial tissue. *J Immunol* 1994;152:6017-27.
28. Soderstrom K, Bucht A, Halapi E, Gronberg A, Magnusson L, Kiessling R. Increased frequency of abnormal $\gamma\delta$ T cells in blood of patients with inflammatory bowel diseases. 1996;156:2331-9.
29. Okada A, Bank I, Rogozinski L, Takihara Y, Mak TM, Chess L, et al. Structure of the $\gamma\delta$ T cell receptor of a human thymocyte clone. *J Exp Med* 1988;168:1481-6.
30. Goodman T, Lefrancois L. Expression of the $\gamma\delta$ T cell receptor on intestinal CD8⁺ intraepithelial lymphocytes. *Nature* 1988;333:855-8.
31. McMenamin C, Pimm C, McKersey M, Holt PG. Regulation of IgE responses to inhaled antigen in mice by antigen specific $\gamma\delta$ T cells. *Science* 1994;265:1869-71.
32. Hyjek EM, Bartkowiak J, Drodz R, Wasik TJ, Jasinski M, Kaneko Y, et al. Evidence for B cell mediated activation of V δ 1⁺ T lymphocytes during progression of HIV infection. *J Immunol* 1997;158:464-74.
33. Hacker G, Kromer S, Falk M, Heeg K, Wagner H, Pfeffer K, et al. V delta 1⁺ subset of human $\gamma\delta$ T cells respond to ligands expressed by EBV-infected Burkitt lymphoma cells and transformed B lymphocytes. *J Immunol* 1992;149:3984.
34. Hacker G, Adam S, Wagner H. Interaction between gamma delta T cells and B cells regulating IgG production. *Immunology* 1995;84:105-10.
35. Wright A, Lee JE, Link MP, Smith SD, Carroll W, Levy R, et al. Cytotoxic T lymphocytes specific for self tumor immunoglobulin express T cell receptor δ chains. *J Exp Med* 1990;169:1557-64.
36. Mami-Chouaib F, DelPorto P, Delorme D, Hercend T. Further evidence for a $\gamma\delta$ T cell receptor mediated TCT.1/CD48 recognition. *J Immunol* 1991;147:2864.
37. Qian D, Sperling AI, Lanci DW, Tatsumi Y, Barrett TA, Bluestone JA, et al. The γ chain of the high affinity receptor for IgE is a major functional subunit of the T-cell antigen receptor complex in $\gamma\delta$ T lymphocytes. *Proc Natl Acad Sci U S A* 1993;90:11875-79.

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