Clonal Th2 lymphocytes in patients with the idiopathic hypereosinophilic syndrome


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Summary. Idiopathic hypereosinophilic syndrome (HES) and Gleich’s syndrome are related disorders characterized by persistent or recurrent hypereosinophilia of unknown origin. Elevated IgE levels and polyclonal hypergamma-globulinaemia are considered as markers of benign outcome in this setting as they are generally associated with predominant cutaneous manifestations and favourable response to glucocorticoid therapy. In a previous study, we identified a clonal population of CD3\(^{-}\)CD4\(^{+}\) Th2-like lymphocytes secreting interleukin (IL)-5 and IL-4 in peripheral blood of a patient fulfilling the diagnostic criteria of HES with associated serum hyper-IgE. We now extend this observation by describing identical findings in three additional patients, and we compare their clinical and biological parameters with five other patients with HES. Chromosomal abnormalities were detected in purified CD3\(^{-}\)CD4\(^{+}\) Th2 cells from three patients, among whom one developed anaplastic null cell lymphoma. We therefore suggest that a careful search for T-lymphocyte clonality and cytogenetic changes should be included in the work-up of HES for adequate management.

Keywords: hypereosinophilic syndrome, lymphoma, IL-5, IgE, CD3\(^{-}\)CD4\(^{+}\).

Idiopathic hypereosinophilic syndrome is defined as persistent hypereosinophilia of unknown origin complicated by organ damage or dysfunction (Fauci et al., 1982; Weller & Bubley, 1994). Although this entity was introduced in an attempt to standardize management of such patients (Chusid et al., 1975), the heterogeneous nature of hypereosinophilic syndrome rapidly emerged with regard to clinical and biological parameters, disease course and response to therapy. Patients with clinical and biological markers reminiscent of myeloproliferative disorders generally responded poorly to glucocorticoids and presented severe cardiac complications of hypereosinophilia. In contrast, high IgE levels and polyclonal hypergamma-globulinemia were considered to be markers of good prognosis as they were frequently associated with restriction of clinical manifestations to the skin and favourable response to corticosteroid therapy (Weller & Bubley, 1994). A similar profile is observed in patients with Gleich’s syndrome (Gleich et al., 1984), a distinct entity in which chronic idiopathic hypereosinophilic syndrome is associated with episodic angioedema.

Interleukin (IL)-5 is a highly specific eosinophilopoietic cytokine which increases eosinophil levels both by stimulating their differentiation from bone marrow precursors and by inhibiting peripheral apoptosis (Her et al., 1991; Wardlaw, 1994; Simon & Blaser, 1995; Walsh, 1997). IL-5 overproduction by Th2-type lymphocytes has been demonstrated in a variety of hypereosinophilic disorders, including parasitic or allergic diseases and Omenn’s syndrome (Sanderson, 1992; Schandené et al., 1993; Romagnani, 1994, 1996). Th2-type cells also secrete IL-4 and IL-13, which are responsible for the hyperproduction of IgE often associated with hypereosinophilia (Punnonen et al., 1997).

We and others have demonstrated the presence of circulating T cells overproducing IL-5 in some patients with idiopathic hypereosinophilia (Cogan et al., 1994; Brugnoni et al., 1996; Kitano et al., 1996; Schandené et al., 1996; Simon et al., 1996, 1999; Bank et al., 1998). T-cell
involvement in the pathogenesis of this disorder is further suggested by the fact that long-term evolution is occasionally complicated by the development of T-cell lymphoma (Weller & Bubley, 1994). This led us to systematically perform T-cell immunophenotyping, a search for peripheral blood T cells in a consecutive series of nine patients with persistent idiopathic hypereosinophilia.

MATERIALS AND METHODS

Patients. Patients with eosinophil levels exceeding 1·5 × 10^9/l were recruited in several Belgian hospitals. Thorough evaluation of hypereosinophilia was performed by local medical teams and medical records were then consulted by our group. Among these patients, nine presented persistent idiopathic hypereosinophilia and were thus selected for further testing in our laboratory. At time of evaluation, patient 5 was under treatment (methylprednisolone 32 mg/d) because of rapid deterioration of heart function and patient 6 had interrupted corticosteroid therapy 1 week before hospitalization. All other patients were free from treatment.

Cell purification. Circulating leucocytes were obtained from patients either by venepuncture in 60-ml heparinized syringes or by cytapheresis (patients 1, 3 and 4) after informed consent and from buffy coats of healthy blood donors. Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation on Lymphoprep (Nycomed, Oslo, Norway) according to the manufacturer’s instructions. After washing with Hank’s balanced salt solution (HBSS) (Gibco, Life Technologies, Paisley, UK), PBMCs were resuspended either with Hank’s balanced salt solution (HBSS) (Gibco, Life Technologies, Paisley, UK), PBMCs were resuspended either in normal culture medium composed of RPMI-1640 (Bio Whittaker/Boehringer Ingelheim) and 40 % FCS or in cold-conservation medium composed of RPMI-1640 (Schering-Plough, Kennelworth, NJ, USA) for immediate testing or in cold-conservation medium composed of RPMI-1640 supplemented with 10 % heat-inactivated fetal calf serum (FCS) (Bio Whittaker/Boehringer Ingelheim) and 40 µg/ml gentamicin (Schering-Plough, Kennelworth, NJ, USA) for immediate testing or in cold-conservation medium composed of RPMI-1640 supplemented with 10 % dimethylsulphoxide (Sigma Chemical, St. Louis, MO, USA) and 10 % FCS for storage in liquid nitrogen before testing.

Purified CD3^-CD4^+ cells were obtained from patients 1, 3, 4 and 9 and CD3^-CD8^-CD4^- cells were obtained from healthy donors by negative selection. PBMCs were thawed and treated with Lymphopak® T (One Lambda, Los Angeles, CA, USA) according to the manufacturer’s instructions. The remaining cells were resuspended in culture medium at 10 × 10^6/ml and were incubated with mouse monoclonal antibodies (mAbs) against CD14, CD19, CD56 and CD8 with or without anti-CD3 (Becton Dickinson, Mountain View, CA, USA) for 30 min at 4°C. After washing with HBSS, cells were resuspended in culture medium and incubated with sheep anti-mouse IgG-coated magnetic Dynabeads (Dynal, Oslo, Norway) for 45 min at 4°C. Coated cells were removed with a magnet, leaving purified CD3^-CD4^+ or CD3^-CD4^- cells in suspension. CD3^-CD4^- cell preparations were > 95 % pure, and CD3^-CD4^- cell preparations contained less than 0·5 % CD3-positive cells and > 95 % CD4^- cells, as assessed by flow cytometry.

Flow cytometry. Flow cytometric analysis of the surface phenotype was performed by two- and three-colour immunofluorescence using fluorescein isothiocyanate (FITC), phycoerythrin (PE)- and peridinin chlorophyll-a protein (PerCP)- conjugated mAbs. Surface antigens of T cells were stained with mAbs against T-cell receptor (TCR)-α/β, TCR-γ/δ, CD3, CD4, CD8, CD7, CD27, CD2, CD28, HLA-DR, CD95 and CD45RO from Becton Dickinson. Data were collected on at least 10 000 viable cells using a FACSScan flow cytometer and CELLQUEST software (Becton Dickinson).

Flow cytometry was also used for the detection of intracytoplasmic cytokine expression in lymphocyte subsets. To this end, total T cells (CD4^- and CD8^-) were isolated from PBMCs of all patients except patient 1 and from healthy control subjects using the same procedure as described above, except that only mAbs against CD14, CD19 and CD56 were used. These cells were incubated at 10^9/ml with Brefeldine A (Sigma Chemical) at 10 µg/ml, alone or combined with 50 ng/ml phorbol 12-myristate 13-acetate (PMA) (Sigma Chemical) and 0·1 µg/ml calcium ionophore A23187 (Calbiochem-Behring, San Diego, CA, USA) for 6 h in culture medium (37°C, 5 % CO2). Surface antigens were stained on aliquots of 2 × 10^6 cells with FITC- or PE-conjugated anti-CD8 mAb and PerCP-conjugated anti-CD3 mAb. Staining of CD8 was preferred to CD4 because of important down-regulation of surface CD4 expression on T cells after in vitro stimulation. Cells were fixed with FACS Lysing Solution (Becton Dickinson) for 10 min at room temperature in the dark, washed and then permeabilized with 0·5 ml FACS Permeabilizing Solution (Becton Dickinson) under the same conditions. Intracellular cytokines were stained with PE- or FITC-conjugated mAbs against IL-4, IL-13, γ-interferon (IFN-γ) (Becton Dickinson) and IL-5 (Pharmingen, San Diego, USA). Negative controls for cytokine expression were provided by unstimulated cells treated only with Brefeldine A and by intracellular staining of stimulated cells with isotype-matched irrelevant PE- or FITC-conjugated mAbs. Triple stain flow cytometry permitted distinct analysis of cytokine expression in gated CD3^-CD8^- (equivalent to CD3^-CD4^+) and CD3^-CD8^- (equivalent to CD3^-CD4^-) cells.

Intracellular expression of CD3-ε and TCR-α/β in CD3^-CD4^- cells from patients 1, 3, 4 and 9 was demonstrated by flow cytometry. PBMCs were incubated with FITC/anti-CD4 mAb and PerCP/anti-CD3 mAb. Cells were washed, fixed and permeabilized as above, and then stained with PE-conjugated anti-CD3 or anti-TCR-α/β mAb. Intracytoplasmic expression of CD3-ε and TCR-α/β was determined after gating on surface CD3^-CD4^- viable lymphocytes.

Immunocytochemical staining of intracytoplasmic CD3-ε antigen. After purification, CD3^-CD4^- cells from patients 1, 3 and 4 were cytospun, fixed and stained with anti-CD3-ε mAb (Dako, Glostrup, Denmark), revealed by the alkaline phosphatase antialkaline phosphatase (APAAP) technique (Dako), before and after permeabilization with acetone at 4°C for 10 min. Stained cells were then visualized by light microscopy.

Evaluation of T-cell clonality. Rearrangement of the β-chain of the TCR was performed by Southern blot analysis using a
DNA probe corresponding to the second constant region of the gene, as previously described (Cogan et al., 1994).

TCR γ-chain gene rearrangement was studied by multiplex polymerase chain reaction (PCR) followed by denaturing gradient gel electrophoresis using primers according to the method described previously by Theodorou et al. (1995).

Stimulation of CD4+ T cells with mitogenic agents in vitro. Purified CD4+ T cells from healthy blood donors and CD3−CD4+ cells from patients 1, 3, 4 and 9 (5 × 10⁵/ml) were stimulated using 1 ng/ml PMA and 0.1 μg/ml calcium ionophore A23187 in culture medium. All culture supernatants were harvested after 48 h at 37°C under 5% CO₂ for measurement of cytokine concentrations.

Determination of cytokine levels and soluble CD25 levels in serum and culture supernatants. Commercial enzyme-linked immunosorbent assay (ELISA) kits were used to determine concentrations of IL-13 and soluble IL-2R-α (sCD25) (Biosource, Camarillo, CA, USA). Other cytokine concentrations were measured by two-site sandwich ELISA using antibodies from Genzyme (Cambridge, MA, USA) for IL-2, Chromogenix (Mölndal, Sweden) for IFN-γ, Mabtech (Stockholm, Sweden) for IL-4 and Pharmingen for IL-5.

RESULTS

Clinical profile and laboratory findings in nine patients with chronic idiopathic hypereosinophilia

In our consecutive series of nine patients with chronic idiopathic hypereosinophilia, eight fulfilled the diagnostic criteria of HES, whereas the clinical and biological findings in patient 4 were consistent with the diagnosis of Gleich’s syndrome. Their major clinical manifestations and biological findings are summarized in Tables I and II. In all cases, allergy was excluded on the basis of clinical history and negative testing for specific IgE against common allergens and serological testing for infection by the human immunodeficiency virus was negative. Thorough diagnostic work-up including radiological evaluation, radioisotopic imaging and search for blastic cells in blood and bone marrow specimens failed to reveal an underlying malignant haematological disorder at the time of diagnosis. Cytogenetic analysis of blood and bone marrow at time of presentation did not disclose chromosomal abnormalities except for three mitoses with breakpoints on chromosome 1 in a bone marrow specimen from patient 1. This patient developed disseminated anaplastic large null cell KI-positive lymphoma 4 years after diagnosis of idiopathic hypereosinophilic syndrome.

T-cell immunophenotyping in patients with idiopathic hypereosinophilic syndrome

Immunophenotypic analysis of circulating lymphocytes revealed the presence of an abnormal CD4+ subset in four patients (patients 1, 3, 4 and 9) characterized by absent surface expression of the CD3 antigen (Fig 1A). These cells which also stained negatively for α/β and γ/δ TCR (not shown) represented 56%, 90%, 84% and 16% of total CD4+ lymphocytes and reached absolute circulating levels of 0.7, 3.61, 2.5 and 0.24 × 10⁹/l in patients 1, 3, 4 and 9.

<table>
<thead>
<tr>
<th>Table I. Clinical profile of patients with chronic idiopathic hypereosinophilia.</th>
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<tr>
<td><strong>Age</strong></td>
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<td>9</td>
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</table>

* Age at appearance of symptoms and discovery of hypereosinophilia.
† Age at time of immunological evaluation.
‡ Small nodules in the parotid region.
GC, glucocorticoids.
The CD3−CD4+ cells expressed CD2, CD28 and the CD45RO isoform characteristic of memory T cells while lacking CD7 and CD27 (Fig 1B). They displayed an activated phenotype, assessed by expression of HLA-DR, and stained positively for CD95/Fas receptor (Fas-R). Intracytoplasmic expression of the CD3- and TCR-α/β chains in CD3−CD4+ cells from patients 1, 3, 4 and 9 was demonstrated by flow cytometry and by immunocytochemical staining using the APAAP method (not shown) after permeabilization of the cells. Distribution of surface antigens on the CD3−CD4+ T-cell population from all four patients was similar to that of CD4+ T cells obtained from normal subjects shown in Fig 1B. Immunophenotyping of lymphocytes from patients 2, 5, 6, 7 and 8 was normal except for a CD4/CD8 ratio of 0.6 in patient 2 (Table III). Furthermore, their CD4+ T cells displayed normal expression of the above-mentioned surface markers (Fig 1B).

**T-cell clonality in patients with idiopathic hypereosinophilic syndrome**

Clonality of circulating T cells was examined on whole blood by Southern blot and PCR analysis of the TCR β- and γ-chain genes respectively. A monoclonal rearrangement pattern was found for three of the patients with an aberrant CD3−CD4+ T-cell population (1, 3 and 4), as well as for patient 6 (see Table III and Fig 2). A germline configuration was observed in peripheral blood obtained from the remaining patients (not shown).

**The CD3−CD4+ T-cell subset is monoclonal and displays a Th2-type profile of cytokine production**

The T-cell clonality detected in peripheral blood of patients 1, 3 and 4 was shown to be restricted to the CD3−CD4+ subset, as demonstrated after purification (see Cogan et al., 1994 for patient 1 and Fig 2A for patients 3 and 4). Furthermore, although the search for T-cell clonality performed on whole blood was negative for patient 9, a clonal rearrangement pattern was clearly observed after purification of the CD3−CD4+ population (Fig 2C).

The cytokine profile of isolated CD3−CD4+ cells was investigated by measuring cytokine concentrations in culture supernatants after 48 h stimulation with PMA.

![Fig 1.](image-url) Identification of a subset of CD3−CD4+ T cells in four patients with chronic idiopathic hypereosinophilia. PBMCs from hypereosinophilic patients and healthy blood donors were stained with FITC-, PE- and PerCP-conjugated mAbs against CD3, CD4, CD7, CD27, CD45RO and HLA-DR and the surface phenotype was analysed by flow cytometry. Results for patients 1, 3, 4 and 9 are illustrated by patient 3, and results for patients 2, 5, 6, 7 and 8 are illustrated by patient 7. After gating on CD4+ lymphocytes (A), flow cytometric analysis revealed a subset of cells lacking expression of CD3 in patient 3, whereas in patient 7 all CD4+ cells stained positively for CD3. Surface phenotype of gated CD3−CD4+ T cells from patient 3 is compared with that of gated CD3−CD4+ T cells from patient 7 and from a healthy blood donor (B).

| Table II. Major laboratory findings in hypereosinophilic patients. |
|---------------------------------|------------------|
| **Patient** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** |
| Total WBC (10^9/l) | 16.76 | 26.8 | 16.9 | 14.8 | 18.7 | 8 | 18.8 | 16.4 | 7.8 |
| Lymphocytes (10^9/l) | 1.68 | 2.57 | 4.63 | 3.42 | 1.32 | 2 | 1.91 | 2.66 | 2.24 |
| IgE (kU/l) | 2.505 | 67 | 340 | 15.64 | < 3.5 | 327 | 4 | 20 | 478 |
| IgG (g/l) | 15.5 | 6.3 | 7 | 10.7 | 6.3 | 14.2 | 11.1 | 12.2 | 19.2 |
| IgM (g/l) | 72 | 3.8 | 3.1 | 12.5 | 2.1 | 2.95 | 1.5 | 1.6 | 2.36 |
| Vitamin B12 (ng/l) | 228 | 287 | 380 | 424 | 975 | 1043 | > 2000 | > 2000 | 1450 |

Normal values: IgE, < 100 kU/l; IgG, 6.5–15 g/l; IgM, 0.4–2.5 g/l; vitamin B12, 180–700 ng/l.
and A23187 calcium ionophore. High levels of IL-4 and IL-5 were detected in supernatants compared with CD3^+CD4^+ T cells from healthy subjects and IFN-γ was virtually absent (Table IV). These cells also secreted IL-2, although to a lesser extent than purified CD4^+ T cells from healthy subjects. Flow cytometric analysis of intracytoplasmic cytokine expression after brief stimulation using PMA and A23187 confirmed the Th2-like profile of CD3^+CD4^+ cells. Indeed, a significant proportion of these cells expressed IL-4 (77% for patient 3, 69% for patient 4, 83% for patient 9), IL-13 (64% for patient 3, 49% for patient 4, 80% for patient 9) and IL-5 (95% for patient 3, 69% for patient 4, 25% for patient 9). These results contrasted with the minute fractions of IL-4-, IL-13- and IL-5-producing cells among normal CD3^+CD4^+ lymphocytes from healthy subjects (1±0.46±0.2% for IL-4, 0.76±0.13% for IL-13 and 0.4±0.14% for IL-5; means ± s.e.m. of cytokine-positive cells in 11 healthy subjects). Patient 9 differed from patients 1, 3 and 4 in that IFN-γ was barely detectable in the CD3^+CD4^+ cells from these patients, whereas it was

<table>
<thead>
<tr>
<th>Patient</th>
<th>Serum IgE (kU/l)</th>
<th>Serum sCD25* (pg/ml)</th>
<th>Serum IL-5† (pg/ml)</th>
<th>Lymphocyte phenotyping</th>
<th>TCR rearrangement</th>
<th>Cytogenetic analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2505</td>
<td>128</td>
<td>320</td>
<td>56% CD3^+CD4^+ cells</td>
<td>C</td>
<td>Chromosome 1‡</td>
</tr>
<tr>
<td>3</td>
<td>340</td>
<td>180</td>
<td>50</td>
<td>90% CD3^+CD4^+ cells</td>
<td>C</td>
<td>Chromosomes 6, 10§</td>
</tr>
<tr>
<td>4</td>
<td>15640</td>
<td>175</td>
<td>&lt; 20</td>
<td>84% CD3^+CD4^+ cells</td>
<td>C</td>
<td>Chromosomes 6, 10§</td>
</tr>
<tr>
<td>9</td>
<td>478</td>
<td>nd</td>
<td>&lt; 20</td>
<td>16% CD3^+CD4^+ cells</td>
<td>C</td>
<td>Normal</td>
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<tr>
<td>2</td>
<td>67</td>
<td>&lt; 16</td>
<td>&lt; 20</td>
<td>CD4/8 ratio 0.6</td>
<td>GL</td>
<td>Normal</td>
</tr>
<tr>
<td>5</td>
<td>&lt; 3.5</td>
<td>&lt; 16</td>
<td>&lt; 20</td>
<td>Normal</td>
<td>GL</td>
<td>Normal</td>
</tr>
<tr>
<td>6</td>
<td>327</td>
<td>&lt; 16</td>
<td>&lt; 20</td>
<td>Normal</td>
<td>C</td>
<td>Normal</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>2506</td>
<td>&lt; 20</td>
<td>Normal</td>
<td>GL</td>
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<td>8</td>
<td>20</td>
<td>nd</td>
<td>&lt; 20</td>
<td>Normal</td>
<td>GL</td>
<td>Normal</td>
</tr>
</tbody>
</table>

* Normal value < 16 pg/ml.
† Normal value < 20 pg/ml.
‡ Bone marrow: three mitoses with breakpoints on chromosome 1.
§ Peripheral blood; partial deletions on chromosomes 6 and 10.
C, monoclonal; GL, germline configuration; nd, not done.

Fig 2. T-cell clonality in peripheral blood of a subgroup of patients with persistent idiopathic hypereosinophilia. (A) PCR analysis of the TCR γ-chain gene rearrangement pattern performed on whole blood from a healthy control subject (lane 1), patient 4 (lane 2) and patient 3 (lane 4) and performed on purified CD3^+CD4^+ T cells from patient 4 (lane 3) and patient 3 (lane 5). (B) Southern blot analysis of the TCR β-chain gene rearrangement pattern in peripheral blood from patient 6 (lane 1) and a healthy control subject (lane 2). (C) PCR analysis of the TCR γ-chain gene rearrangement pattern in purified CD3^+CD4^+ cells (lane 2) from patient 9 compared with a negative control (lane 1). G, germinal bands; R, rearranged bands.
produced by 11.4% of CD3^+CD4^+ cells from patient 9 (compared with 19.5 ± 2.3% as mean ± s.e.m. of IFN-γ-positive cells among total CD3^+CD4^+ cells in 11 healthy subjects). The cytokine profile of CD3^+CD4^+ lymphocytes from all patients, including those with a clonal CD3^+CD4^+ subset, was similar to that of normal CD4^+ T cells from healthy subjects (not shown).

**DISCUSSION**

We have identified four patients presenting persistent idiopathic hypereosinophilia with an underlying T-cell disorder characterized by monoclonal expansion of an aberrant CD4^+CD3^- T-cell population producing high levels of IL-5, IL-4 and IL-13 indicative of a Th2 profile. We compared the clinical and biological features of these patients with those of five other patients fulfilling the diagnostic criteria of HES in whom no aberrant T-cell subset was detected. We observed a homogeneous profile among those with clonal CD3^+CD4^+ Th2 cells consisting in predominance of cutaneous manifestations (including pruritus, eczema and urticaria) with lack of severe end-organ involvement, elevated serum IgE levels and polyclonal hypergammaglobulinaemia involving either IgM or IgG. Although vitamin B12 is classically considered as a marker of the myeloproliferative variant of HES, we observed elevated serum levels in one patient with T-cell clonality. Moreover, serum levels of IL-5 and soluble CD25, two potential markers of T-cell activation, were of no help in the discrimination of patients with an underlying clonal T-cell disorder in our series. In this regard, it is now established that eosinophils also produce IL-5 (Warland, 1994; Lamkhioued et al, 1996) and are a potential source of soluble CD25 (Aldebert et al, 1994).

The CD3^+CD4^- cells were likely to be involved in the development of hypereosinophilia, through the production of IL-5, and increased serum IgE levels, through the production of IL-4 and IL-13. However, their role in the nature of the clinical manifestations remains unknown. It has been shown that the CD4^+CD45RO^+CD7^- subset of T cells preferentially produces Th2-type cytokines (Autran et al, 1995) and it has been suggested that it may represent a population of specialized skin-related memory T cells (Legac et al, 1992; Baars et al, 1995; Reinhold et al, 1996). Thus, the CD3^-CD4^+(CD45RO^-CD7^-) cells may be responsible for the predominant development of cutaneous manifestations in our patients.

Since our first observation (Cogan et al, 1994), several groups have identified a circulating clone of IL-5-producing T cells in patients with persistent idiopathic hypereosinophilia (Brugnoni et al, 1996; Kitano et al, 1996; Simon et al, 1996, 1999). Recently, in a series of 60 patients with chronic hypereosinophilic disease, Simon et al (1999) reported the occurrence of an underlying T-cell disorder characterized by an aberrant surface phenotype in 16 cases. Our own series confirms that three-colour flow cytometric analysis of surface markers on peripheral blood lymphocytes is a critical step in the identification of hypereosinophilic patients with clonal Th2 cells and indicates that particular attention should be paid to the presence of CD3^-CD4^+ T cells. However, Southern blot and PCR analysis of TCR gene rearrangement patterns should also be performed systematically on peripheral blood and bone marrow in order to detect T-cell clonality in patients with a normal flow cytometric profile, as in the case of patient 6. In addition, for patients who do present a lymphocyte population with an aberrant phenotype, these investigations should also be performed after purification of the abnormal cells, especially when they represent only a small proportion of total lymphocytes. Indeed, although a subset of CD3^-CD4^+ cells was detected by flow cytometry in peripheral blood from patient 9, a clonal rearrangement pattern of the TCR could only be demonstrated after purification of these cells.

The pathogenesis of T-cell clonality in the setting of chronic hypereosinophilic disease remains unknown. Simon et al (1999) showed that the aberrant T-cell population lacked CD95/Fas-R expression in 8/16 patients of their series and demonstrated that deficient Fas-mediated apoptosis was involved in expansion of a CD3^-CD4^-CD8^- IL-5-producing lymphocyte subset in one such patient (Simon et al, 1996). However, CD3^-CD4^+ cells from our patients expressed Fas-R and underwent apoptosis upon engagement of this receptor (unpublished observations), suggesting that primary events leading to clonal T-cell expansion can differ.

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**Table IV. Cytokine profile of clonal CD3^-CD4^+ cells.**

<table>
<thead>
<tr>
<th>Patient</th>
<th>IL-5 (ng/ml)</th>
<th>IL-4 (ng/ml)</th>
<th>IL-2 (ng/ml)</th>
<th>IFN-γ (U/ml)</th>
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<tbody>
<tr>
<td>1</td>
<td>46.75</td>
<td>54.54</td>
<td>750</td>
<td>&lt; 20</td>
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<td>3</td>
<td>14.47</td>
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<td>160</td>
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<td>4</td>
<td>5.47</td>
<td>2.91</td>
<td>172</td>
<td>&lt; 20</td>
</tr>
<tr>
<td>9</td>
<td>24.65</td>
<td>nd</td>
<td>172-3</td>
<td>nd</td>
</tr>
<tr>
<td>Control^†</td>
<td>0.5 ± 0.07</td>
<td>0.67 ± 0.17</td>
<td>2177 ± 391</td>
<td>1066 ± 289</td>
</tr>
</tbody>
</table>

* Purified CD3^-CD4^+ cells from patients 1, 3, 4 and 9 or CD3^-CD4^+ cells from three healthy subjects (control) were incubated at 5 × 10^7/ml with PMA (1 ng/ml) and A23187 calcium ionophore (0.1 µg/ml) for 48 h and culture supernatants were harvested for determination of cytokine concentrations by sandwich ELISA.

† Results are expressed as means of three subjects ± s.e.m.

nd, not done.
among hypereosinophilic patients with an underlying T-cell disorder. The homogeneous profile of the aberrant T cells in our patients suggests a common pathogenic agent. The absence of surface TCR/CD3 expression could be consistent with viral infection of the T cells, as lymphotropic viruses such as human immunodeficiency virus (HIV)-1 (Willard-Gallo et al., 1990), human T-cell lymphocytotropic virus (HTLV)-I (de Waal Malefyt et al., 1990), and human herpesvirus (HHV)-6 (Furukawa et al., 1990) virus (HTLV)-I (Willard-Gallo et al., 1990). Monotherapy with type I IFNs for patients with clonal IL-5-producing T cells could be detrimental despite their suppressive effects both on eosinophils (Aldebert et al., 1996; Morita et al., 1996) and on IL-5 production by lymphocytes (Schandene et al., 1996). Indeed, recent studies in our laboratory have demonstrated a potent antiapoptotic effect of IFN-α on clonal CD3−CD4− cells from our patients in vitro (unpublished observations). Future proposals for the management of hypereosinophilic patients such as ours should be based on knowledge concerning the activation pathways operating in these cells and should be designed to target both T-cell expansion and Th2 cytokine production.

NOTE ADDED IN PROOF

Since submission of the manuscript, patient 3 has developed a lymphomatous mass in the cervical region consisting of CD3−CD4+ cells.

ACKNOWLEDGMENTS

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