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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 hypergammaglobulinaemia 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 hypergammaglobulinaemia 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 hypereosinophilia 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

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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 T-cell clonality and analysis of cytokine production by peripheral blood T cells in a consecutive series of nine patients with persistent idiopathic hypereosinophilia.

MATERIALS AND METHODS

Patients. Patients with eosinophil levels exceeding $1\cdot5 \times 10^9$/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 in normal culture medium composed of RPMI-1640 (Bio Whittaker/Boehringer Ingelheim, Verviers, Belgium) 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 $CD3^+CD4^+$ cells were obtained from healthy donors by negative selection. PBMCs were thawed and treated with Lymphokwik 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 FACScan 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 10^6/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% CO_2). Surface antigens were stained on aliquots of 2×10^5 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 (5×10^5/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% CO_2 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 K1-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 $0{\cdot}24 \times 10^9$/l in patients 1, 3, 4 and 9

Table I. Clinical profile of patients with chronic idiopathic hypereosinophilia.

Patient	Age		Sex	Clinical manifestations	Evolution
	Diagnosis*	Evaluation†			
1	30	30	M	Prurigo, dyspnoea, weight loss, digital thrombotic vasculitis	Initial response to combined GC and IFN-α; anaplastic null cell lymphoma (after 4 years)→dead
2	62	65	M	Pruritus, diarrhoea, cholestasis, weight loss	Favourable response to combined hydroxyurea and GC
3	16	20	F	Eczema, subcutaneous nodules‡, tenosynovitis, weight loss	No effect of GC or IFN-α on $CD3^-CD4^+$ subset; favourable response to fludarabine
4	21	21	F	Eczema, urticaria, episodic angioedema	Favourable clinical response and regression of $CD3^-CD4^+$ subset with GC (prednisolone 10 mg/d)
5	36	36	F	Diffuse pulmonary infiltrate, grade 4/4 mitral valve regurgitation, weight loss	Valve replacement → embolic stroke (died 4 months after diagnosis)
6	20	30	F	Eczema, urticaria	Favourable clinical and biological response to GC
7	27	28	M	Mitral valve regurgitation due to chordal rupture, weight loss	Favourable clinical and biological response to combined hydroxyurea and GC
8	34	34	M	Dyspnoea, splenomegaly, weight loss	Initiation of hydroxyurea
9	47	58	F	Urticaria	No treatment

* Age at appearance of symptoms and discovery of hypereosinophilia.
† Age at time of immunological evaluation.
‡ Small nodules in the parotid region.
GC, glucocorticoids.

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
Eosinophils	6·12	16·62	8·92	9·1	3·99	3·66	9·15	11·7	2·97
Lymphocytes	1·68	2·57	4·63	3·42	1·32	2	1·91	2·66	2·24
IgE (kU/l)	2505	67	340	15640	< 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.

respectively. Furthermore, 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-ε 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

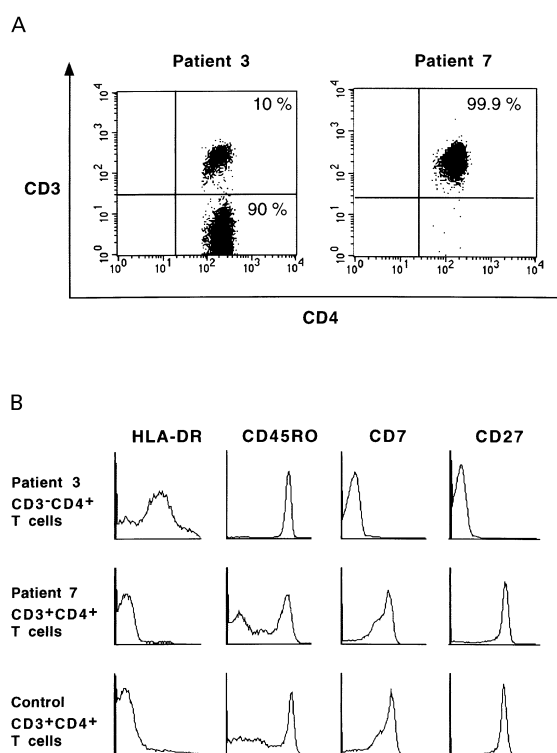

Fig 1. Identification of a subset of $CD3^-CD4^+$ T cells in four patients with chronic idiopathic hypereosinophilia. PBMCs from hypereosinophilic patients and healthy blood donors were stained with FITC-, PE- and PerCP-conjugated mAbs against CD3, CD4, CD7, CD27, CD45RO and HLA-DR and the surface phenotype 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 III. Overview of T-cell investigations in patients with chronic idiopathic hypereosinophilia.

Patient	Serum IgE (kU/l)	Serum sCD25* (pg/ml)	Serum IL-5† (pg/ml)	Lymphocyte phenotyping	TCR rearrangement	Cytogenetic analysis
1	2505	128	320	56% $CD3^{-}CD4^{+}$ cells	C	Chromosome 1‡
3	340	180	50	90% $CD3^{-}CD4^{+}$ cells	C	Chromosomes 6, 10§
4	15640	175	< 20	84% $CD3^{-}CD4^{+}$ cells	C	Chromosomes 6, 10§
9	478	nd	< 20	16% $CD3^{-}CD4^{+}$ cells	C	Normal
2	67	< 16	< 20	CD4/8 ratio 0·6	GL	Normal
5	< 3·5	< 16	< 20	Normal	GL	Normal
6	327	< 16	< 20	Normal	C	Normal
7	4	2506	< 20	Normal	GL	Normal
8	20	nd	< 20	Normal	GL	Normal

* 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.

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 (1·46 ± 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

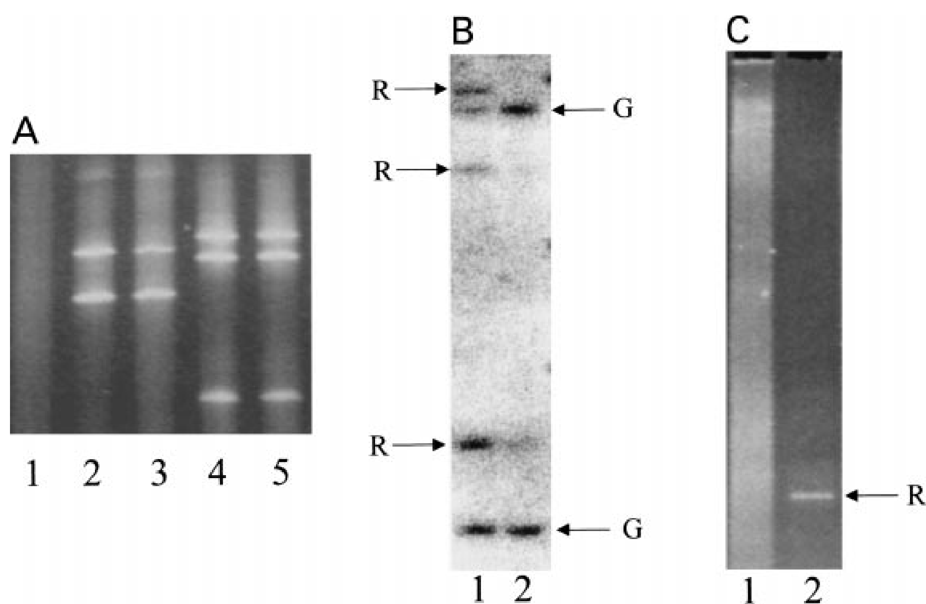

Fig 2. T-cell clonality in peripheral blood of a subgroup of patients with persistent idiopathic hypereosinophilia. (A) PCR analysis of the TCR γ-chain gene rearrangement pattern performed on whole blood from a healthy control subject (lane 1), patient 4 (lane 2) and patient 3 (lane 4) and performed 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.

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

Patient	IL-5 (ng/ml)	IL-4 (ng/ml)	IL-2 (ng/ml)	IFN-γ (U/ml)
1	46·75	54·54	750	< 20
3	14·47	6·38	799	160
4	5·47	2·91	172	< 20
9	24·65	nd	172·3	nd
Control†	0·5 ± 0·07	0·67 ± 0·17	2177 ± 391	1066 ± 289

* Purified $CD3^-CD4^+$ cells from patients 1, 3, 4 and 9 or $CD3^+CD4^+$ cells from three healthy subjects (control) were incubated at 5×10^5/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.

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 (Wardlaw, 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

among hypereosinophilic patients with an underlying clonal 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 lymphocytotrophic virus (HTLV)-I (de Waal Malefyt *et al*, 1990) and human herpesvirus (HHV)-6 (Furukawa *et al*, 1994) have been shown to down-regulate transcription of specific CD3 chains. Although HTLV-I and HTLV-II proviral sequences were not detected in clonal $CD3^{-}CD4^{+}$ cells from patients 1, 3 and 4 and antibodies to TAX were not detected in their serum, involvement of other lymphotropic viruses is currently under investigation.

Detection of an IL-5-producing clonal T-cell population in patients with persistent idiopathic hypereosinophilia has prognostic implications as several investigators have reported protracted development of T-cell lymphoma in this setting. In some cases, a $CD3^{-}CD4^{+}$ lymphocyte population was detected before progression towards lymphoid malignancy (O'Shea *et al*, 1987; Bagot *et al*, 1990; Moraillon *et al*, 1991; Simon *et al*, 1999). Importantly, Simon *et al* (1999) observed that 3/16 hypereosinophilic patients with an aberrant T-cell population developed T-cell lymphoma that conserved the abnormal surface phenotype initially observed, suggesting that the aberrant cells could be the precursors of malignant T cells. In another study, lymphomatous cells from a patient presenting cutaneous T-cell lymphoma associated with hypereosinophilia produced Th2-type cytokines and bore a $CD3^{-}CD4^{+}$ phenotype (Brugnoni *et al*, 1997). The malignant potential of the $CD3^{-}CD4^{+}$ cell population in our patients is suggested by the development of anaplastic null cell lymphoma in patient 1 4 years after presentation and by the appearance of chromosomal abnormalities consisting in partial deletions on chromosomes 6 and 10 in the $CD3^{-}CD4^{+}$ cells from patients 3 and 4 after 4 and 2 years' disease progression respectively. Repeated cytogenetic analysis of blood and marrow specimens may help in the identification of patients with clonal IL-5-producing T cells at risk for the development of a malignant disorder. Interestingly, similar cytogenetic changes have been reported in lymphomatous cells from patients with Sezary syndrome (Limon *et al*, 1995), which shares a number of features with our patients' T-cell disorder (Pancake *et al*, 1995, 1996). Both diseases are characterized by the presence in peripheral blood of clonal T cells (Weinberg *et al*, 1995) that display a mature $CD4^{+}CD45RO^{+}CD7^{-}$ phenotype (Wood *et al*, 1990; Bogen *et al*, 1996) and a Th2-type cytokine profile (Vowels *et al*, 1992). However, weak or absent CD3 expression on Sezary cells is uncommon (Bogen *et al*, 1996; Sano *et al*, 1998).

Finally, the T-cell-mediated nature of this hypereosinophilic disease should be taken into consideration for patient management. Corticosteroids remain useful for patients with an underlying T-cell disorder through their dual suppressive action on eosinophils and T-cell cytokine production (Wallen *et al*, 1991; Schleimer & Bochner, 1994; Umland *et al*, 1997). However, although patients 1 and 3 did respond to corticosteroid treatment in terms of clinical manifestations and eosinophil levels, the aberrant T-cell population remained unaffected. In contrast, the clonal population in patient 4 rapidly declined from 84% to 7% of total $CD4^{+}$ T cells after initiation of corticosteroid treatment. Although IFN-α has been used with success in the management of patients with idiopathic hypereosinophilic syndrome (Bockenstedt *et al*, 1994 Butterfield & Gleich, 1994), 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 (Schandené *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.

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