

F. Milazzo
S. Piconi
D. Trabattoni
C. Magni
M. Coen
A. Capetti
M.L. Fusi
C. Parravicini
M. Clerici

Intractable pruritus in HIV infection: immunologic characterization

Authors' affiliations:

F. Milazzo, S. Piconi, C. Magni, M. Coen,
A. Capetti, Divisione di Malattie Infettive,
Ospedale L. Sacco, Milan
D. Trabattoni, M.L. Fusi, M. Clerici, Cattedra di
Immunologia, Università degli Studi di Milano,
Padiglione L.I.T.A., Ospedale L. Sacco, Milan
C. Parravicini, Cattedra di Anatomia Patologica,
Ospedale L. Sacco, Milan, Italy

Correspondence to:

Mario Clerici, MD
Cattedra di Immunologia
Padiglione L.I.T.A.
Ospedale L. Sacco
Via G.B. Grassi 74
20157 Milan
Italy

Key words: cytokines; dermatology; HIV; IgE; immunology; pruritus.

Background: Severe, intractable pruritus, often associated with erythematopapular skin lesions and hypereosinophilia, is a condition observed in some nonatopic, HIV-infected patients. We performed immunovirologic analyses of this condition.

Methods: Immunologic (mitogen-stimulated production of cytokines, tumor necrosis factor- α [TNF- α], and soluble CD23; serum levels of soluble CD23, ICAM-1, TNF- α , IgG, IgE, and IgA) and virologic (HIV viral load) parameters were analyzed in six patients with therapy-resistant pruritus. Hypereosinophilia was present in all these patients. Results were compared to those of seven HIV-seropositive individuals similar to the first one in terms of CD4 counts and clinical staging, but without pruritus.

Results: Hypereosinophilia; hyper-IgE and hyper-IgA; augmented interleukin (IL)-4, IL-5, and sCD23; and reduced interferon- γ production by mitogen-stimulated peripheral blood mononuclear cells (PBMC) were detected when patients with pruritus were compared to HIV controls. HIV viral load was also augmented in patients in whom pruritus was present.

Conclusions: The results suggest that therapy-resistant, intractable pruritus accompanied by hypereosinophilia may be used to define a subset of HIV-seropositive individuals showing prototypic hyperactivation of humoral immunity, and in whom augmented HIV viral load is present.

Date:

Accepted for publication 21 September 1998

To cite this article:

Milazzo F., Piconi S., Trabattoni D., Magni C., Coen M.,
Capetti A., Fusi M.L., Parravicini C. & Clerici M.
Intractable pruritus in HIV infection: immunologic
characterization.
Allergy 1999; 54, 266–272.

Copyright © Munksgaard 1999

ISSN 0105-4538

Severe pruritus unresponsive to symptomatic treatment and appearing in nonatopic patients is a relatively common feature of advanced HIV infection. Alterations in the number of eosinophils can cause pruritus, and these two conditions are often associated (1–5) in this disease. Augmented serum concentrations of IgE may also be observed in HIV-infected patients (6) and hyper-IgE appear-

Table 1. Characterization of six HIV-seropositive subjects with intractable pruritus (1–6) and seven (7–13) clinically comparable HIV-seropositive subjects without pruritus (HIV control)

Patient no.	Age (years)	Sex	Risk factor	CDC	WBC / μ	Lymphocytes / μ l	Granulocytes / μ l	Eosinophils / μ l	CD4+ / μ l	CD8+ / μ l	B cells / μ l	NK cells / μ l	HIV viral load copies/ml
1	32	M	IVDU†	IVC1	2770	620	1150	378	6	248	10	36	414 558
2	35	M	IVDU	IVC2	2020	470	840	545	7	259	10	119	312 578
3	39	M	IVDU	IVC1	3220	1020	660	773	6	795	15	21	399 541
4	31	M	IVDU	III	3100	850	1400	443	10	221	28	125	555 215
5	39	M	IVDU	II	2300	360	1350	496	117	493	112	45	212 369
6	57	M	Homo	IVB	2600	570	1230	468	12	323	20	142	378 984
7	35	M	IVDU	II	3400	1100	1750	31	17	105	6	28	239 750
8	38	M	IVDU	II	1000	230	650	16	81	424	63	108	128 577
9	31	F	IVDU	IVC1	1600	340	1040	48	21	179	12		404 332
10	37	M	Homo	IVC2	4300	450	3030	204	132	715	33	88	288 788
11	30	F	Homo	IVC1	1600	352	1120	30	5	157	4	43	121 451
12	34	M	Homo	IVC1	3100	217	2573	133	4	85	6	183	239 425
13	34	M	IVDU	IVC1	2400	620	2950	38	20	286	56	9	141 255

†IVDU: intravenous drug user.

ing in HIV-infected nonatopic patients has been correlated with faster decline in CD4 counts and more rapid disease progression in both adult and pediatric HIV infection (7–10).

Defective production of type-1 cytokines (interleukin [IL]-2, IL-12, and interferon-gamma [IFN- γ]) and augmented production of type-2 cytokines (IL-4, IL-5, and IL-10) was also suggested by some authors to be present in HIV infection (11) and was proposed as a possible *in vitro* immunologic marker of progression (11). IL-4 is responsible for the isotype switch from IgG to IgE (12); IL-5 induces IgA secretion from plasma cells and the proliferation and differentiation of eosinophils (13). IgE production is also stimulated by other factors such as sCD23, a molecule derived from the low-affinity IgE receptor (14–16). That these factors are tightly regulated is suggested by the observations that IL-4 augments the concentration of sCD23 (17–19), and in turn sCD23 regulates the production of other cytokines such as IFN- γ and tumor necrosis factor- α (TNF- α) (20). Consequently, sCD23 also functions as a proinflammatory factor, as it activates antigen-specific and antigen nonspecific lymphocytes secondarily to the stimulation of such cytokines (21).

We analyzed immunologic and virologic parameters in HIV-infected patients characterized by the presence of intractable pruritus and multiple erythematopapular lesions, and in HIV-seropositive subjects comparable to the first group in terms of both CD4 counts and clinical stage, but who did not suffer from pruritus. The results suggested that the presence of intractable pruritus was associated with

exacerbated hyperactivation of humoral immunity and, possibly, with higher HIV viral load.

Material and methods

Patient selection

Thirteen HIV-infected patients followed by the I Divisione di Malattie Infettive, Ospedale L. Sacco, Milan, Italy, were selected for this study. Six of the patients had been suffering for at least 6 months from severe itching resistant to antihistamine (oxatomide 20 mg twice daily and/or cetirizine 10 mg/day for 1 month; subsequently, idoxazine 25 mg twice daily plus topical betamethasone) and cortisone (dexamethasone 16 mg/day), and showed the presence of multiple erythematopapular lesions in the upper body and hypereosinophilia. Seven patients comparable to the first ones in terms of clinical stage and CD4 counts, but without either pruritus or skin lesions, were selected as controls (HIV control). Clinical and epidemiologic characterization of the 13 patients is shown in Table 1. All patients were screened for parasite infestations by microscopic examination of stool specimens, and for presence of allergic reactions to common antigens, and were negative for both factors. To avoid possible allergies to drugs, all therapies were suspended for at least 1 week before the study period. None of the patients were treated with recombinant colony-stimulating factors (e.g., GM-CSF).

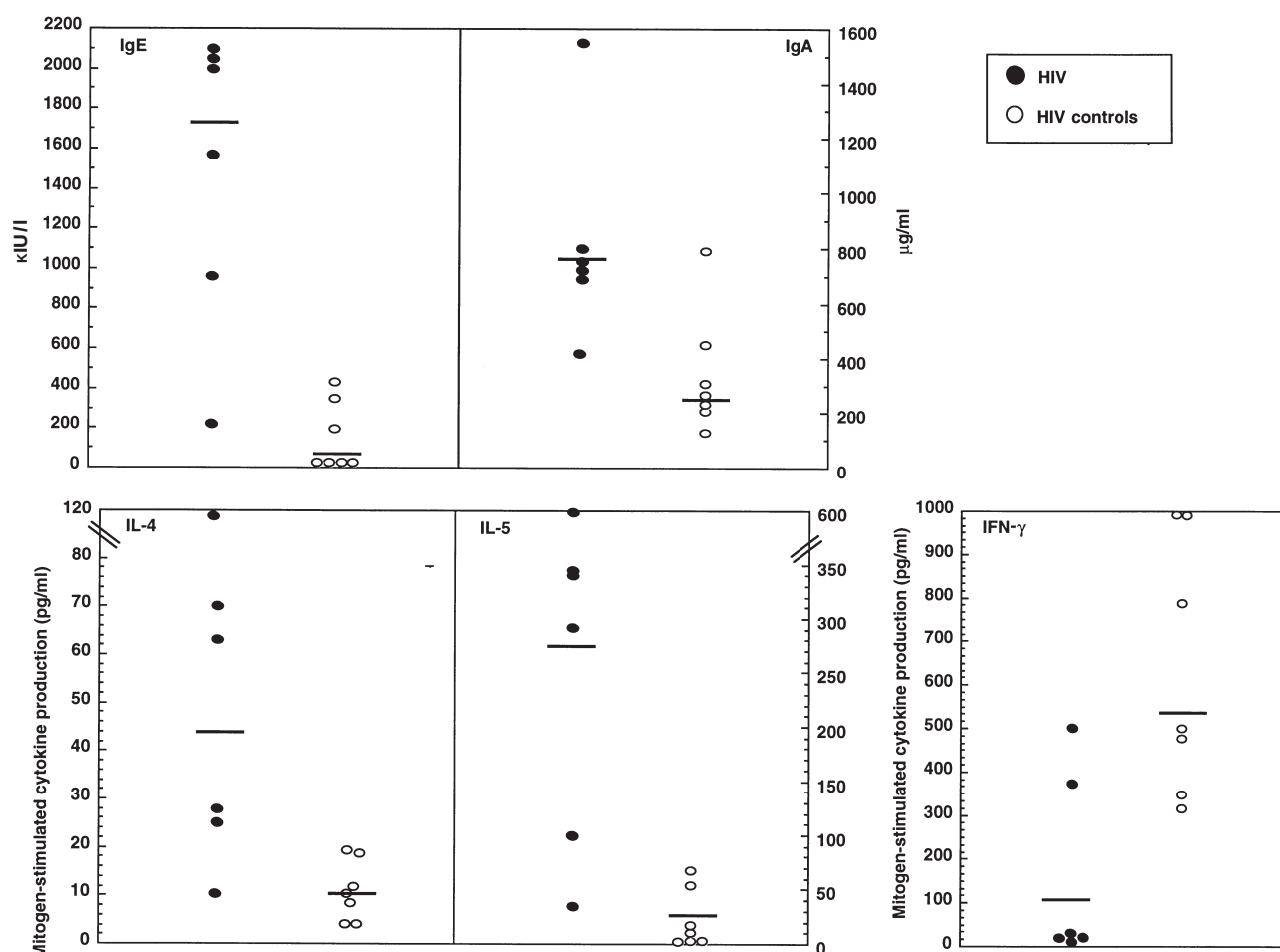


Figure 1. Upper panels: IgE (left panel) and IgA (right panel) serum concentrations in six HIV-infected patients with severe, intractable pruritus (HIV) (●) and in seven HIV-infected patients without pruritus (HIV control) (○). Mean values are indicated by horizontal line. Lower panels: mitogen-stimulated production of IL-4 (left panel), IL-5 (middle panel), and IFN- γ (right panel) in six HIV-infected patients with severe, intractable pruritus (HIV) (●) and in seven HIV-infected patients without pruritus (HIV control) (○). Mean values are indicated by horizontal line.

Finally, none of the patients were receiving antiretroviral drugs at the time the study was performed (four individuals – two in each group – were antiretroviral drugs-naïve; therapy had been suspended for at least 3 months in the other patients).

HIV viral load

Branched DNA analyses were performed with a commercially available branched DNA assay (Chiron Diagnostics Ltd, Halstead, UK).

Skin biopsies

Biopsies of the lesional skin were performed in all the patients with pruritus at the beginning of this study; ethical reasons prevented biopsy of the HIV-control group. Skin punch biopsies were fixed in formalin and embedded in

paraffin. Serial sections were partly stained with HE, Giemsa, and PAS for routine histopathology and partly processed for immunohistochemistry. Antibodies against CD4 (clone OPD4), CD8 (clone OPD8), CD68 (clone Mac 387), and CD20 (clone L26), all from DAKO (Glostrup, Denmark), were employed, and the reaction was revealed with a double indirect peroxidase method and DAB as chromogenic substrate.

Blood sample collection

Whole blood was collected in vacutainer tubes containing EDTA (Becton Dickinson, Rutherford, NJ, USA). PBMC were separated on lymphocyte separation medium (Organon Teknika Corp., Durham, NC, USA) and washed twice in PBS. The samples were kept at room temperature and used within 2 h.

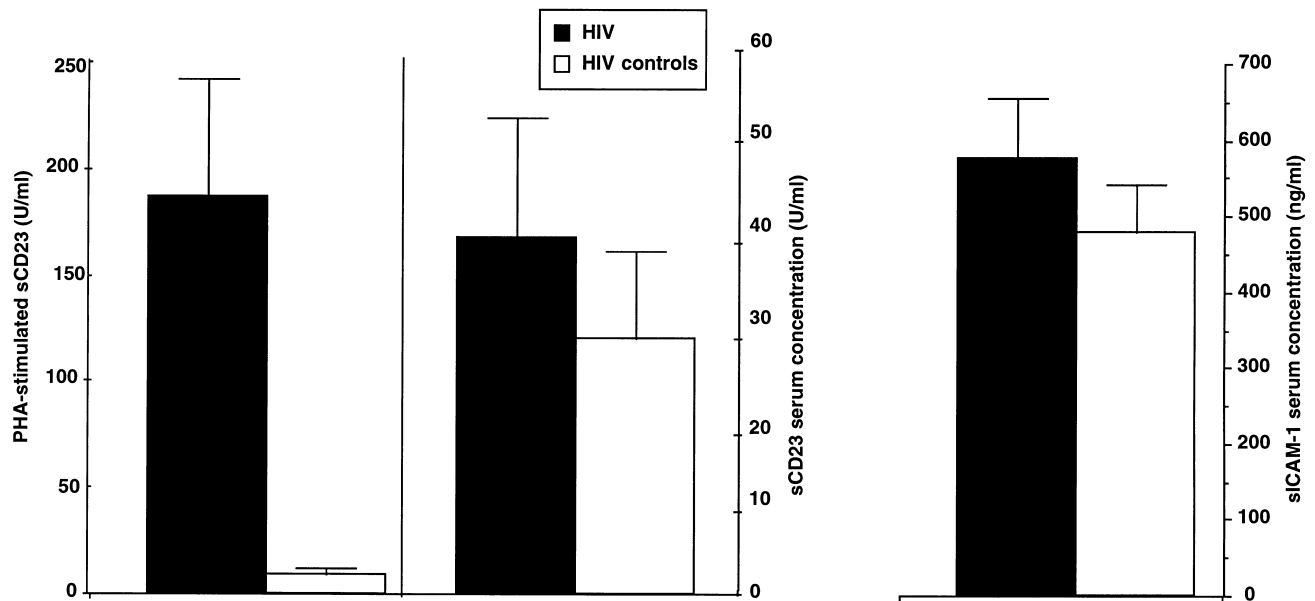


Figure 2. Mitogen-stimulated sCD23 production (left panel), sCD23 (center panel), and sICAM-1 (left panel) serum concentrations in six HIV-infected subjects with severe, intractable pruritus (HIV) (■) and in seven HIV-infected patients without pruritus (HIV control) (□). Mean and standard deviations are shown.

Serum immunoglobulin concentration

Serum concentration of IgG and IgA was determined by immunoturbidimetry (Specific Instruments, Kone, Helsinki, Finland) after antigen-antibody complex formation (Orion Antibodies, Helsinki, Finland). Serum IgE was measured by immunocapture (Pharmacia CAP System IgE RIA, Uppsala, Sweden). IgE values are expressed as kilo International Units per liter (kIU/l). The interassay coefficients of variation were less than 10%.

In vitro cytokine production

PBMC, resuspended at 3×10^6 /ml in RPMI 1640, were either unstimulated, or stimulated for 48 h with PHA (1:100) (IFN- γ , IL-4, IL-5) or LPS (SIGMA Diagnostics, St Louis, MO, USA) (1 μ g/ml for 48 h) (TNF- α). The cultures were supplemented with 5% pooled AB⁺ human serum (SIGMA Diagnostics). Cytokine production was evaluated with commercially available ELISA assays as follows: IFN- γ : human Intertest-gamma (Genzyme, Cambridge, MA, USA); IL-4: human Intertest-4 (Genzyme); IL-5: human IL-5 ELISA kit (Immunotech, Marseille, France); TNF- α : human Intertest TNF- α (Genzyme), following the procedures suggested by the manufacturers. Values for all the cytokines were calculated from a standard curve of the corresponding recombinant human cytokine.

Measurement of sCD23 in serum and supernatant

sCD23 was measured in sera and in PHA-stimulated culture supernatants (see above) with commercially available ELISA (CD23 Test Kit; T Cell Diagnostic, Cambridge, MA, USA).

Measurement of TNF- α and sICAM-1 in serum

Serum concentration of TNF- α and sICAM-1 was measured with commercially available ELISA kits (human TNF- α Intertest [Genzyme] and ICAM-1 Test [T Cell Diagnostic], respectively).

Results

Skin biopsies

Skin biopsies, performed on lesional skin in all the HIV-infected patients with pruritus, showed a perivascular inflammatory infiltrate containing mononuclear cells and eosinophils in the papillary and upper reticular dermis. Most mononuclear cells were CD8+, while CD4+ lymphocytes and CD20+ B cells were usually absent. CD68+ monocytes were inconspicuous (data not shown).

Hematologic and virologic parameters

Serum eosinophils were significantly augmented in patients compared to HIV controls, whereas no differences were observed when the absolute numbers of lymphocytes, neutrophils, and granulocytes were analyzed in the two groups (Table 1). Analogously, similar amounts of CD4+ and CD8+ T cells, B cells, and natural killer (NK) cells were observed in the two groups of patients (Table 1). Serum concentration of IgE was increased in patients compared to HIV controls ($P=0.002$) (Fig. 1). Serum concentrations of IgA were similarly augmented in patients with intractable pruritus, even if the differences did not reach statistical significance (Fig. 1), whereas the concentration of IgG was similar in patients and HIV controls.

Mitogen-stimulated cytokine production

PHA-stimulated IL-4 and IL-5 production was significantly augmented, and IFN- γ production was significantly reduced in patients compared to HIV controls (IL-4: $P=0.01$; IL-5: $P=0.006$; IFN- γ : $P=0.03$) (Fig. 1). Serum concentrations of these cytokines were similarly altered, although not significantly (IL-4: patients: 1.4 pg/ml; controls: 0.5 pg/ml; IL-5: patients: 4.9 pg/ml; controls: 2.2 pg/ml; IFN- γ : patients: 1.5 pg/ml; controls: 6.7 pg/ml). Finally, both mitogen-stimulated and serum concentrations of TNF- α were augmented in patients compared to HIV controls even if the differences did not reach statistical significance (patients: 7.1 pg/ml; controls: 2.3 pg/ml). Thus, a diminished production of IFN- γ is associated with an increase in IL-4 and IL-5 production in HIV-infected individuals suffering from severe pruritus.

Serum concentration and mitogen-induced production of sCD23

Mitogen-stimulated sCD23 production was increased in HIV-infected patients with intractable pruritus ($P=0.003$) (Fig. 2). Serum concentration of sCD23 was increased as well, although not significantly so (Fig. 2). Because it was suggested that sCD23 favors secretion of IgE and production of TNF- α , we analyzed the data in a linear regression model. The results of the analysis showed the presence in patients with pruritus, but not in HIV controls, of a positive correlation between serum concentration of sCD23 and IgE ($r=0.66$, $P<0.1$). The results of this analysis also showed a negative correlation between serum concentrations of sCD23 and TNF- α ($r=-0.8$, $P<0.05$) (a similar trend was observed between mitogen-stimulated sCD23 and TNF- α production) ($r=-0.64$, $P=0.078$).

Serum concentration of sICAM-1

sICAM-1 serum concentration was augmented, although not significantly so, in patients with intractable pruritus compared to HIV controls (Fig. 2). Because sCD23 increases the expression of ICAM-1 by monocyte-enriched cell cultures, this result could be explained by the previously mentioned augmentation of sCD23 detected in our patients.

HIV viral load

HIV viral load was augmented, although not significantly so, in patients with intractable pruritus compared to HIV controls. Thus, mean ($\pm$ SD) HIV viral load was $378\,874 \pm 46\,517$ copies/ml in patients with pruritus and $223\,368 \pm 38\,934$ copies/ml in the HIV controls (patients were not receiving antiretroviral therapy at the time of the study).

Discussion

HIV infection is frequently associated with dermatologic diseases among which severe and therapy-resistant pruritus is relatively common (5). We investigated more in depth a series of immune parameters in HIV-infected individuals suffering from intractable pruritus; the results suggest that this symptom might define a group of HIV-infected individuals in whom prototypic hyperactivation of humoral immunity and augmented HIV viral load are observed.

An imbalance in type-1 and type-2 cytokines was found to be present in HIV infection (11). A further, although still controversial, observation suggested that this imbalance might be associated with disease progression (11). Immunologic analyses of patients with intractable pruritus showed the production of IL-4 and IL-5 (type-2 cytokines) to be augmented, and that of IFN- γ (a type-1 cytokine) to be reduced compared to clinically similar patients not suffering from pruritus. That altered cytokine production causes a deep impairment of the immune response in HIV-seropositive individuals with intractable pruritus was highlighted by the observation that serum concentrations of both IL-4-driven IgE and of IL-5-stimulated IgA were augmented in these individuals. The possible biologic importance of these immunologic alterations was underlined by results showing that HIV viral load is augmented in these patients. Taken together these results further support the hypothesis that in HIV infection:

- 1) hyper-IgE and hypereosinophilia are associated with the worst prognosis
- 2) alterations in the type-1/type-2 cytokine profile are prognostically unfavorable.

Biopsies of the lesional skin showed the presence of endothelial inflammation in association with intractable pruritus; this inflammation could explain the etiopathogenesis of the symptom. In an attempt to define more accurately the factor(s) involved in this inflammatory process, we investigated serum levels and mitogen-stimulated production of sCD23, a molecule capable of triggering inflammatory processes via the liberation of various interleukins and monokines (20, 21). sCD23 is the cleaved soluble form of CD23 (the low-affinity receptor for IgE) which is involved in IgE-mediated enhancement of immune response and is expressed on the surface of different cell types (14–16). IL-4 augments the concentration of sCD23 (17, 19); in turn, sCD23 stimulates IgE secretion (17–19) and the production of both TNF- α and IFN- γ . Mitogen-stimulated sCD23 production, as well as serum concentration of sCD23, was augmented in HIV-infected patients with pruritus, and a positive correlation was detected between serum concentration of IgE and sCD23. Our results also included an unexpected finding, as a negative correlation was observed between sCD23 and both TNF- α and IFN- γ mitogen-stimulated production. This could be explained by the observation that sCD23-induced TNF- α production is downmodulated by IL-4 and IL-10 (21) (which were

augmented in our patients). Because the inflammatory properties of sCD23 are mainly thought to be secondary to the stimulation of TNF- α and IFN- γ (20, 21) but neither TNF- α nor IFN- γ was augmented in our patients, these results show that augmented concentrations of sCD23 are observed in HIV-infected individuals independently of TNF- α and IFN- γ secretion. Our data thus suggest that dysregulation of sCD23 is associated with modifications of cytokine production in the inflammatory process underlying intractable pruritus in HIV infection.

The results of our analyses confirm previous results (9) and they extend the immunologic characterization of intractable pruritus in advanced HIV infection. Thus, we believe intractable pruritus in HIV infection to be the clinical manifestation of a complex dysregulation of the immune response in which a nonspecific skin endothelial inflammation and hyperactivation of the humoral response are observed. Because a possible correlation was observed in our patients between intractable pruritus and augmented HIV viral load, the data suggest that the presence of this symptom in HIV-infected patients should stimulate more in-depth analyses and, perhaps, a more aggressive therapeutic approach.

Acknowledgment This paper was supported by grants from Istituto Superiore di Sanita "IX Progetto AIDS 1996" and "Progetto Terapia Antivirale AIDS n. 192003".

References

1. Fenandez C, Ribera M, Bananco JC, Clotet B, Còrenzo JC. Eosinophilic pustular folliculitis in patients with acquired immunodeficiency syndrome. *Int J Dermatol* 1992;**31**:193–195.
2. Drabick JS, Magill AJ, Smith KS, Nutman TB, Benson P. Hypereosinophilic syndrome associated with HIV infection. *South Med J* 1994;**87**:525–529.
3. Smith KS, Skelton HG, Drabick JJ, McCarthy WF, Wagner KF. Hypereosinophilia secondary to immunodysregulation in patients with HIV-1 disease. *Arch Dermatol* 1994;**130**:119–121.
4. Cohen AJ, Steigbigel PT. Eosinophilia in patients infected with human immunodeficiency virus. *J Infect Dis* 1996;**174**:615–618.
5. Carr A, Cooper DA, Penny R. Allergic manifestations of human immunodeficiency virus (HIV) infection. *J Clin Immunol* 1991;**11**:55–64.
6. Lin RY. Chronic diffuse dermatitis and hyper-IgE in HIV infection. *Acta Derm Venereol* 1988;**68**:486–491.
7. Wright DN, Nelson RP, Ledfort DK. Serum IgE and human immunodeficiency virus (HIV) infection. *J Allergy Clin Immunol* 1990;**85**:445–452.
8. Lucey DK, Zajac RA, Melchers GP, Boswell RN. Serum IgE levels in 622 plasmas with human immunodeficiency virus infection: IgE elevations with marked depletion of CD4+ T cells. *AIDS Res Hum Retroviruses* 1990;**6**:127–129.
9. Paganelli R, Scala F, Mezzaroma I, et al. Immunologic aspects of hyperimmunoglobulinemia E-like syndrome in patients with AIDS. *J Allergy Clin Immunol* 1995;**95**:995–1003.
10. Viganò A, Principi N, Crupi L, Onorato J, Vincenzo ZG, Salvaggio A. Elevation of IgE in HIV-infected children and its correlation with the progression of disease. *J Allergy Clin Immunol* 1995;**95**:627–632.
11. Clerici M, Shearer GM. A Th1→Th2 switch is a critical step in the etiology of HIV Infection. *Immunol Today* 1993;**14**:107–111.
12. Pene J, Rousset F, Briere F, et al. IgE production by normal human lymphocytes is induced by interleukin 4 and suppressed by interferons gamma and alpha and prostaglandin E₂. *Proc Natl Acad Sci U S A* 1988;**85**:6880–6884.

13. Stavnezer J. Immunoglobulin class switching. *Curr Opin Immunol* 1996;**8**:199–205.
14. Lederman S, Yellin MJ, Krichevsky A, Belko J, Lee JJ, Chess L. Identification of a novel surface protein on activated CD4+ T cells that induces contact-dependent B cell differentiation (help). *J Exp Med* 1992;**175**:1091–1101.
15. Delespesse G, Sarfati M. IgE-binding factor (soluble CD23) and IgE regulation. *Res Immunol* 1992;**141**:75–77.
16. Richards ML, Katz DH. Biology and chemistry of the low affinity IgE receptor (Fc ϵ RII/CD23). *Crit Rev Immunol* 1991;**11**:65–86.
17. Conrad DH, Keegan AD, Kalli KR, van Dusen R, Rao M, Levine AD. Superinduction of low affinity IgE receptors on murine M lymphocytes by lipopolysaccharides and IL-4. *J Immunol* 1988;**141**:1091–1097.
18. Vercelli D, Jabara B, Lee W, Woodland N, Geha RS, Leng DYM. Human recombinant IL-4 induces Fc ϵ RII/CD23 on normal human monocytes. *J Exp Med* 1988;**167**:1406–1410.
19. Chomarat P, Briolay J, Banchereau J, Miossec P. Increased production of soluble CD23 in rheumatoid arthritis, and its regulation by interleukin-4. *Arthritis Rheum* 1993;**36**:234–239.
20. Armant M, Ishihama IA, Rubio M, Delespesse G, Sarfati M. Regulation of cytokine production by soluble CD23: costimulation of interferon γ secretion and triggering of tumor necrosis factor γ release. *J Exp Med* 1994;**180**:1005–1010.
21. Armant M, Rubio M, Delespesse G, Sarfati M. Soluble CD23 directly activates monocytes to contribute to the antigen-independent stimulation of resting T cells. *J Immunol* 1995;**155**:4868–4875.