

INVESTIGATIVE REPORT

Role of Th2 Cytokines, RANTES and Eotaxin in AIDS-associated Eosinophilic Folliculitis

PAOLO AMERIO¹, ROBERTO VERDOLINI², GIANLUCA PROIETTO³, CLAUDIO FELICIANI³, PAOLA TOTO³, GULNAR SHIVJI¹, FRANCESCO LOCONSOLE⁴, NICOLETTA CASSANO⁴, PIERLUIGI AMERIO⁵, GINO VENA⁴ and DANIEL N. SAUDER¹

¹Division of Dermatology, Sunnybrook Health Science Centre, University of Toronto, Toronto, Canada, ²Department of Dermatology, University of Ancona, Ancona, Italy, ³Department of Dermatology, University of Chieti, Chieti, Italy, ⁴Department of Dermatology, University of Bari, Bari, ⁵Department of Dermatology, University of Sacred Heart, Rome, Italy

The pathogenesis of AIDS-associated eosinophilic folliculitis is still unknown. The expression of chemokines and Th2-type cytokines is increased in other conditions associated with tissue eosinophilia and in allergic reactions. We evaluated the mRNA expression by reverse transcriptase polymerase chain reaction of two Th2 cytokines (interleukin-4 and interleukin-5) and of two chemokines (RANTES and eotaxin) in the skin of 6 patients with AIDS-associated eosinophilic folliculitis; the tissue localization of eotaxin was shown by immunohistochemistry. We demonstrated the increased expression of interleukin-4, interleukin-5, RANTES and eotaxin in lesional skin of the patients compared to normal skin of HIV+ individuals. We concluded that a Th2 pattern is present in AIDS-associated eosinophilic folliculitis. The cytokine milieu in this disease may favour a Th2 immune response to an unknown antigen, whereby RANTES and eotaxin act in synergy with interleukin-4 and interleukin-5 to mediate tissue inflammation. Key words: AIDS; chemokines; eosinophilic folliculitis pathogenesis.

(Accepted January 17, 2001.)

Acta Derm Venereol 2001; 81: 92–95

Paolo Amerio, V^a Divisione, I.D.I. IRCCS, Via dei Monti di Creta 104, IT-00167 Rome, Italy. E-mail: p.amerio @idi.it

AIDS-associated eosinophilic folliculitis (AIDS-EF) is a pruritic papular dermatosis occurring in HIV-infected individuals, first described in 1986 (1). This disease was at first thought to be a clinical variant of the eosinophilic pustular folliculitis of Ofuji but now it is considered to be a distinct entity (2). The clinical and histopathological features of AIDS-EF have been well delineated (3) and include: a chronic pruritic, follicular and papular eruption involving face, scalp and upper trunk, sparing the palms and soles. This disease has a histological picture similar to that of Ofuji disease with folliculocentric inflammatory infiltrate with predominance of eosinophils and lymphocytes. The diagnosis is based on clinical findings, histological examination and negative cultures to rule out potential infectious aetiologies (4).

The pathogenesis is still controversial. According to some authors, AIDS-EF may be an autoimmune reaction towards a sebaceous gland component (5), a second hypothesis is based on the assumption that AIDS-EF could be linked to an abnormal immunological reaction to microbial or parasitic infection (6, 7) which induces an eosinophilic response persisting even after the clearance of the pathogen from the skin.

Dysregulation towards a predominant Th2 cytokines expression (IL-4 and IL-5) has been implicated in diseases characterized by eosinophilic activation, such as parasitic infection, as well as atopic or allergic diseases (8). It has been suggested that Th2 cytokines play a role in Ofuji disease (9), a disease which is closely related to AIDS-EF. IL-5 mediates blood eosinophilia (10) and in the tissue it could prolong mature eosinophils survival and stimulate their proinflammatory functions (11). Recently, it has been shown that this cytokine exerts its effects on eosinophils, enhancing the chemotactic effects of chemokines such as regulated upon activation normal T cells secreted (RANTES) and eotaxin (12, 13).

Chemokines are chemotactic molecules that can be subdivided into several subclasses on the basis of the cystein residues position. The α -chemokines (CXC) include IL-8 and IP-10, the β -chemokines (CC) include RANTES, eotaxin and monocyte chemotactic proteins (MCPs); more recently 2 other families have been included (14). RANTES is a CC chemokine that mediates chemotaxis and activation of many cell types and is important, in the skin, in the recruitment of eosinophils in the allergic late phase reaction. High levels of RANTES expression in skin have been associated with a Th2 type of cytokine production in allergic atopic dermatitis (15). Eotaxin is a potent chemoattractant of the CC family. It is a chemotactic agent for eosinophils, basophils, mast cells, T helper type 2 lymphocytes (16) and a major mediator of allergic responses. Its chemotactic activity is 100 times more potent than RANTES over eosinophils. Many cell types have been found to produce eotaxin predominantly after cytokines stimulation (16, 17) but little is known about its expression in human skin.

To better understand the role of cytokines and chemokines in AIDS-EF, we evaluated the expression of mRNA of two Th 2 type cytokines, IL-4 and IL-5, and two chemokines, RANTES and eotaxin, in lesional skin of 6 patients affected by AIDS-EF. Moreover we evaluated, with an immunohistochemistry technique, the cellular source of eotaxin.

MATERIAL AND METHODS

Clinical characteristics

Six patients with the characteristic clinical and pathological features of AIDS-EF were examined. Some of their clinical features are listed in Table I. Skin biopsies from all patients confirmed the diagnosis of AIDS-EF. Bacterial cultures and histological analysis with special stains for micro-organisms were negative.

RNA extraction

Three-mm punch biopsies were performed on all patients, snap frozen in liquid nitrogen and stored at -80°C until use. As a control normal skin samples were obtained from HIV-positive subjects without EF. Total RNA extracted from each sample, using the acid guanidinium thiocyanate-phenol-chloroform, Chomczynsky & Sacchi method (18). The amount of total RNA was measured by a fluorometer (model 450, Sequoia Turner, Mountain view, CA).

Reverse transcriptase polymerase chain reaction (RT-PCR)

Reverse transcription was performed by standard methods previously described (19). Briefly, $1\ \mu\text{g}$ of total RNA was dissolved in $10\ \mu\text{l}$ of water containing 10 U of RNasin (Boeringer Mannheim, Dorval, Quebec, Canada) and $1\ \mu\text{l}$ of oligo dT-primers (Pharmacia, Laval, Quebec, Canada) and was heated to 65°C for 5 min then chilled on ice. The solution was mixed with RT buffer final concentration 60 mM KCl, 50 mM Tris (pH 8.3), 3 mM MgCl₂, 10 mM dithiothreitol (DTT), 1 mM of each dNTP, and 200 U of Moloney murine leukemia virus reverse transcriptase (Gibco, Life Technologies, Burlington, Ontario, Canada) to a total volume of $20\ \mu\text{l}$. After 1 h incubation at 37°C , the reaction was stopped by heating at 95°C for 5 min. Primers sets and positive DNA template were purchased from Dalton Chemicals, York, Ontario, Canada. The primer sets were as follows:

IL-4: sense 5'-CTTCCCCTCTGTTCTTCCT-3'; antisense 5'-TTCTGTGCGAGCCGTTTCCAG-3'
 IL-5: sense 5'-ATGAGGATGCTTCTGCATTTG-3'; antisense 5'-TCAACTTTCATTATCCACTCGGTGTTTCCATTAC-3'
 RANTES: sense 5'-CCTCTCCACAGGTACCAT-3'; antisense 5'-CCTCAGGTTCAAGGACTC-3'
 Eotaxin: sense 5'-CCCAACCACCTGCTGCTTTAACCTG-3'; antisense 5'-TGGCTTTGGAGTTGGAGATTTTGG-3'
 G3PDH: sense 5'-TGAAGGTCGGAGTCAACGGATTTGGT-3'; antisense 5'-CATGTGGGCCATGAGGTCCACCAG-3'

Positive cDNA template for eotaxin was reverse transcribed from RNA extracted from cultured human fibroblasts stimulated for 24 h with recombinant human TNF- α as described previously (19). For the PCR amplification of the cDNA, $2\ \mu\text{l}$ of the cDNA reaction mixture of each sample was added to $8\ \mu\text{l}$ of PCR mix containing $3.3\ \mu\text{l}$ DEPC treated water, $1\ \mu\text{l}$ of $10\times$ PCR buffer, $0.5\ \mu\text{l}$ of each primer (1.25 mM), $1.6\ \mu\text{l}$ of dNTPs, $1\ \mu\text{l}$ of tetramethylammoniumchloride (TMAC) and $0.1\ \mu\text{l}$ of Taq polymerase (Pharmacia, Laval, Quebec, Canada). The mixture was overlaid with $15\ \mu\text{l}$ of mineral oil (Sigma, Oakville, Ontario, Canada). PCR amplification was performed using a Perkin-Elmer Cetus Thermal Cycler 450 (Perkin-Elmer/Cetus, Norwalk, CT, USA) with denaturation for 1 min at 94°C , annealing for 1 min at 60°C , extension for 1 min at 72°C for the housekeeping gene G3PDH, IL-4, RANTES and IL-5. While for the eotaxin primer the conditions were those described previously (17). Eotaxin PCR analysis was only possible on 4 subjects due to limited cDNA quantity. PCR products were run on a 1% agarose gel containing $0.5\ \mu\text{g}/\text{ml}$ ethidium bromide.

Immunohistochemistry

After informed consent, punch biopsies were obtained, from lesional skin of AIDS-EF patients. The specimens were immediately frozen in liquid nitrogen and preserved at -80°C until used. Consecutive cryostat $5\ \mu\text{m}$ sections were cut from each biopsy, air-dried and fixed

in 100% acetone. While the first slides were stained with haematoxylin-eosin to have a morphological control, the following consecutive sections were immunohistochemically investigated with anti-eotaxin antibody. Sections were washed in tris-buffered saline, coated with non-immune goat serum (DAKO, Carpinteria, CA, USA) or isotypic mouse serum (DAKO, Carpinteria, CA, USA) for 20 min and then washed again and incubated for 60 min with the anti-eotaxin monoclonal antibody (R&D system, Minneapolis, MN, USA) at 1:80 dilution. For the revelation system slides were processed following manufacturer's instructions using LSAB+ alkaline phosphatase kit (DAKO, Carpinteria, CA, USA). Briefly, sections were incubated, after a 10 min wash, for 15 min with a mix of biotinylated link antibodies against mouse, rabbit and goat. After a gentle wash, slides were covered with the streptavidin alkaline phosphatase complex and incubated for 15 min, rinsed again and revealed with the substrate chromogen solution (new fuchsin), counterstained with Mayer's haematoxylin, clarified and finally mounted using glycerin medium.

RESULTS

As shown in Table I, all patients had CD4 counts equal to or below 300. Two Th2 cytokines were analysed in lesional skin of AIDS-EF patients and in the normal skin of HIV-positive patients without AIDS-EF. In the normal skin of HIV-positive individuals, we found levels of expression of Th2 cytokines, such as IL-4 and IL-5, below the level of detection. This was the case also for RANTES. Lesional skin of all 6 patients with AIDS-EF showed elevated IL-4 mRNA expression. In these patients, IL-5 mRNA was also elevated but to a lesser extent than IL-4 (Fig. 1). RANTES mRNA was also highly expressed in all AIDS-EF lesional skin tested as was eotaxin mRNA (Fig. 2).

The immunohistochemical analysis performed on a late lesion revealed that eotaxin was strongly expressed by the cellular infiltrate in the abscesses. When the staining was performed in an early lesion in which the follicles were still not completely destroyed, eotaxin was expressed among the specialized mesenchyma in the inflammatory infiltrate around the adnexia and in the papilla among the epithelial cells (Fig. 3).

DISCUSSION

Little is known about the pathogenic mechanism underlying AIDS-EF. As with other eosinophilic diseases, we hypothesize that a predominant Th 2 pattern could be present. We found elevated IL-4, IL-5, RANTES and eotaxin mRNA in patients with AIDS-EF skin lesions.

It has been suggested that IL-4 increases eotaxin production in atopy and in infections (20). Our report is the first demonstrating the coexpression of this chemokine and of Th2 type cytokines in lesional skin of AIDS-EF. The imbalance of Th1 and Th2 cytokines patterns is probably important in AIDS pathogenesis (21). AIDS disease progression seems to be

Table I. Clinical characteristic of patients and laboratory findings

Age/sex	HIV duration (years)	Location of lesion	CD4/mm ³	Associated diseases
32/M	5	Trunk	230	Genital condyloma, hepatitis B
38/M	6	Face, trunk	300	-
40/M	4	Face, trunk	300	Molluscum contagiosum
44/M	3	Trunk	280	-
49/M	3	Face, trunk	270	Hepatitis B
51/F	7	Trunk, arms	250	Hepatitis B and C, endometrial carcinoma

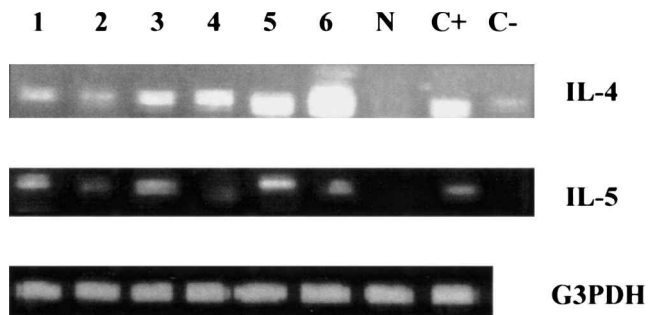


Fig. 1. RT-PCR showing expression of IL-4 and IL-5 mRNA in AIDS-EF lesion samples (lane 1–6) but not in normal HIV+ skin (N). Positive control (C+) was positive DNA template. The house-keeping gene *G3PDH* showed the same intensity in all samples tested. Negative control (C–) was PCR buffer without cDNA.

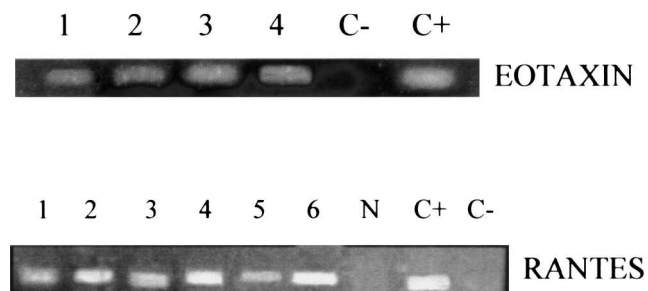


Fig. 2. RT-PCR showing expression of RANTES and eotaxin mRNA in AIDS-EF lesion samples but not in the normal skin of HIV+ patients (N). Positive control (C+) was positive DNA template. Negative control (C–) was PCR buffer without cDNA.

linked to a shift towards a Th2 type of cytokine production (22) or to a decline in Th1 cytokines (23). The clinical finding that AIDS-EF generally occurs in patients with an advanced state of disease progression (CD4+ count of ≤ 300 or less) supports the hypothesis of a preferential Th2 hyperresponsiveness in AIDS-EF lesional skin.

We suggest that AIDS leads to an immune dysregulation in which an imbalance towards Th2 cytokines causes systemically eosinophilia, a condition frequently reported in AIDS patients (24). Triggering by an unknown agent might induce an over-expression of Th2 cytokines in the skin of predisposed AIDS patients, eventually leading to the clinical expression of AIDS-EF. Some reports and open clinical trials suggest that the most probable agent in triggering this aberrant Th2 response is *Demodex folliculorum* (25), although we did not find this pathogen in the microscopic examination of our specimens.

The elevated IL-4 expression that we found in the skin could be the stimulus for the production of eotaxin (20). RANTES and eotaxin produced within AIDS-EF lesions may act in synergy with IL-5 to mediate eosinophil infiltration, probably through upregulation of adhesion molecules. The mechanism of eosinophils recruitment could be maintained even after clearing of the triggering agent, with the production of new eotaxin and RANTES leading to the chronic perpetuation of the disease.

Further studies on the role of other Th2 and Th1 type cytokines in AIDS-EF lesions are needed to fully elucidate the mechanism of eosinophil migration in this disease and to

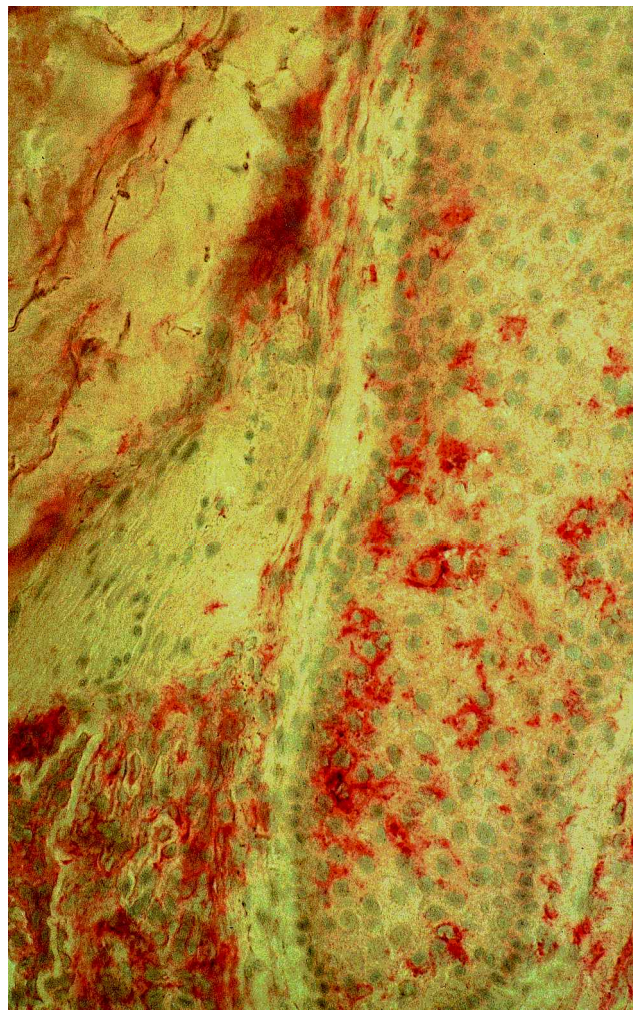


Fig. 3. Strong positivity for eotaxin is present among epithelial cells of the follicle and in the specialized mesenchyma surrounding the outer sheath during an initial phase of the disease (frozen section 250 \times).

indicate the possible triggering agent. Understanding the cytokine dysregulation involved in the pathogenesis of this disease would, hopefully, lead to the development of a more specific treatment.

REFERENCES

1. Soeprono FF, Schimella RA. Eosinophilic pustular folliculitis in patients with acquired immunodeficiency syndrome. *J Am Acad Dermatol* 1986; 14: 1020–1022.
2. Rosenthal D, LeBoit PE, Klumpp L, Berger TG. Human immunodeficiency virus associated eosinophilic folliculitis. A unique dermatosis associated with advanced human immunodeficiency virus infection. *Arch Dermatol* 1991; 127: 206–209.
3. McCalmont TH, Altermus D, Maurer T, Berger TG. Eosinophilic folliculitis. The histologic spectrum. *Am J Dermatopathol* 1995; 17: 439–446.
4. Simpson-Dent S, Fearfield LA, Staughton RC. HIV-associated eosinophilic folliculitis differential diagnosis and management. *Sex Transm Infect* 1999; 75: 291–293.
5. Fearfield LA, Rowe A, Francis N, Bunker CB, Staughton RC. Itchy folliculitis and human immunodeficiency virus infection:

- clinicopathological and immunological features, pathogenesis and treatment. *Br J Dermatol* 1999; 141: 3–11.
6. Brenner S, Wolf R, Ophir J. Eosinophilic pustular folliculitis : A sterile folliculitis of unknown cause? *J Am Acad Dermatol* 1994; 31: 210–212.
 7. Ferrandiz C, Ribera M, Barranco JC, Cloter B, Lorenzo JC. Eosinophilic pustular folliculitis in patients with acquired immunodeficiency syndrome. *Int J Dermatol* 1992; 31: 193–195.
 8. Romagnani S. Regulation of the development of type 2 T-helper cells in allergy. *Curr Opin Immunol* 1994; 6: 838–846.
 9. Fushimi M, Tokura Y, Sachi Y, Hashizume H, Sudo H, Wakita H, *et al.* Eosinophilic pustular folliculitis effectively treated with recombinant interferon gamma : suppression of mRNA expression of interleukin 5 in peripheral blood mononuclear cells. *Br J Dermatol* 1996; 134: 766–772.
 10. Satoh T, Sun L, Li MS, Spry CJ. Interleukin-5 mRNA levels in blood and bone marrow mononuclear cells from patients with the idiopathic hypereosinophilic syndrome. *Immunology* 1994; 83: 308–312.
 11. Foster PS, Hogan SP, Ramsay AJ, Matthaehi KI, Young IG. Interleukin deficiency abolishes eosinophilia, airways hyperactivity, and lung damage in a mouse asthma model. *J Exp Med* 1995; 183: 195–201.
 12. Schweizer RC, Welmers BA, Raaijmakers JA, Zanen P, Lammers JW, Koenderman L. RANTES- and interleukin-8-induced responses in normal human eosinophils: effects of priming with interleukin-5. *Blood* 1994; 83: 3697–3704.
 13. Rafeul A. Chemokine and allergic inflammation. *J Aller Clin Immunol* 1997; 99: 273–278.
 14. Luster AD. Chemokines: chemotactic cytokines that mediate inflammation. *N Engl J Med* 1998; 338: 436–445.
 15. Yamada H, Izutani R, Chihara J, Yodate T, Matsukura M, Tezuka T. RANTES mRNA expression in skin and colon of patients with atopic dermatitis. *Int Arch Allergy Immunol* 1996; 111 Suppl 1: 19–21.
 16. Gutierrez-Ramon JC, Lloyd C, Gonzalo JA. Eotaxin : from an eosinophilic chemokine to a major regulator of allergic reactions. *Immunol Today* 1999; 20: 500–504.
 17. Bartels J, Schuler C, Richter E, Noso N, Kulke R, Christophers E, Schroder JM. Human dermal fibroblast express eotaxin: molecular cloning, mRNA expression, and identification of eotaxin sequence variants. *Biochem Biophys Res Comm* 1996; 225: 1045–1051.
 18. Chomczynsky P, Sacchi N. Single step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. *Annal Biochem* 1987; 162: 156–159.
 19. Kono T, Kondo S, Pastore S, Shivji GM, Tomai MA, McKenzie RC, Sauder DN. Effects of a novel topical immunomodulator, imiquimod, on keratinocyte cytokine gene expression. *Lymphokine Cytokine Res* 1994; 13: 71–76.
 20. Mochizuki M, Bartels J, Mallet AI, Christophers E, Schroder JM. IL-4 induces eotaxin: a possible mechanism of selective eosinophil recruitment in helminth infection and atopy. *J Immunol* 1998; 160: 60–68.
 21. Clerici M, Shearer GM. The Th1-Th2 hypothesis of HIV infection: new insights. *Immunol Today* 1994; 15: 575–581.
 22. Klein SA, Dobmeyer JM, Dobmeyer TS, Pape M, Ottmann OG, Helm EB, *et al.* Demonstration of the Th1 to Th2 cytokine shift during the course of HIV-1 infection using cytoplasmic cytokine detection on single cell level by flow cytometry. *AIDS* 1997; 11: 1111–1118.
 23. Empson M, Bishop GA, Nightingale B, Gasia R. Atopy, anergic status, and cytokine expression in HIV-infected subjects. *J Allergy Clin Immunol* 1999; 103: 833–842.
 24. Cohen AJ, Steigbeigel RT. Eosinophilia in patient infected with human immunodeficiency virus. *J Infect Dis* 1996; 174: 615–618.
 25. Majors MJ, Berger TG, Blauvelt A, Smith KJ, Turner ML, Cruz PD. HIV-related eosinophilic folliculitis: a panel discussion. *Sem Cut Med Surg* 1997; 16: 219–223.