

Evaluation of IgE-sensitization to fungi in HIV-positive patients with eczematous skin reactions

Dorte Nissen, MSc*; Hendrik Nolte, MD, PhD†; Henrik Permin, MD, PhD*; John Heinig, MD‡; Per Stahl Skov, MD, PhD‡; and Svend Norn, PhD§

Background: Human immunodeficiency virus infection is associated with declining immune function and polyclonal B-cell activation leading to elevated IgE-levels. In selected patient categories, increased total IgE may be associated with allergic diseases. Furthermore, a significant number of patients with low CD4+ cell numbers have various skin manifestations, eg, eczema and dermatophytosis. Patients with chronic fungal infections and a tendency to produce increased levels of specific IgE may become allergic and IgE-mediated mechanism may contribute to inflammatory reactions in the skin.

Objective: This study investigates IgE-sensitization of patients infected with human immunodeficiency virus to a panel of fungal extracts of *Candida albicans*, *Fusarium moniliforme*, *Penicillium notatum*, *Pityrosporum ovale*, and *Trichophyton rubrum*.

Methods: Fifteen HIV-positive patients with eczematous skin manifestations and five non-atopic healthy controls were evaluated by basophil histamine release and skin prick test with fungal extracts. The extracts were separated by sodium dodecylsulfate-polyacrylamide gel electrophoresis under reducing conditions and analyzed by IgE-immunoblotting with sera from the patients and controls.

Results: Thirteen of 15 patients (87%) released histamine to one or more of the fungi. Skin prick test was positive to one or more fungi in 7 (47%) patients. Patient sera revealed binding to a wide range of IgE-binding components present in the fungal extracts. The IgE response was most often directed against a 46-kD main protein in the *Candida albicans* extract. There was no correlation between total serum IgE, CD4+ cell counts, and frequency of IgE-sensitization to fungi.

Conclusion: The human IgE response in HIV-infected patients appears to be polyspecific and may be directed against various fungi of which *Candida albicans* may be an important allergen. It is possible that the sensitization is due to frequent infections with *Candida albicans* in this patient population. No unspecific fungal reactions were noted among control patients. These results suggest that allergen-specific IgE-mediated mechanism may contribute to the pathogenesis of the eczematous skin reaction in HIV-infected patients.

Ann Allergy Asthma Immunol 1999;83:153-159.

INTRODUCTION

Infection with human immunodeficiency virus (HIV) induces a decline in

the immune function because of a progressive depletion of helper T-lymphocytes. This decline results in opportunistic infections with microorganisms that are part of the environment and the patients' own microflora, eg, the skin flora.

Patients infected with HIV show a high prevalence of atopic diseases

(IgE-mediated reactions).¹⁻⁴ This might be due to decreased cellular immunity and a change or switch in cytokine production to a Th2 like profile leading to a polyclonal activation of B cells and increased IgE production.⁵⁻⁸ Furthermore, skin manifestations that resemble atopic dermatitis are often observed. This indicates clinical and immunologic similarities in the pathogenesis of the skin reactions of patients with HIV and atopic dermatitis.^{2,8}

Recently, we demonstrated that atopic dermatitis patients are sensitized and may react clinically to a range of fungi that inhabits greasy skin areas and mucosal surface.⁹ The same fungi (*Pityrosporum ovale*, *Candida albicans*, *Penicillium notatum*, *Fusarium moniliforme*, and *Trichophyton rubrum*) have been reported to infect HIV-patients,¹⁰⁻¹⁵ especially *Candida albicans*.¹⁶ We hypothesize that HIV-infected patients may become sensitized to these fungi and display IgE-mediated skin reactions like patients with atopic dermatitis.

The aim of this study was to examine a selected group of HIV-infected patients with reduced immune function and eczematous skin manifestations for the presence of IgE antibodies by basophil histamine release, and skin prick test towards a panel of fungi. Two different preparations of fungal extracts were obtained, ie, a cellular extract and a culture filtrate extract containing mainly extra-cellular components. Generally, fungal extracts are inadequately characterized and standardized extracts are lacking; thus, further analysis by IgE-immunoblotting was performed to identify the IgE-binding components of the fungi in these preparations.

* Department of Infectious Diseases, National University Hospital, Copenhagen, Denmark.

† Department of Internal Medicine, National University Hospital, Copenhagen, Denmark.

‡ Department of Allergy, National University Hospital, Copenhagen, Denmark.

§ Institute of Pharmacology, University of Copenhagen, Copenhagen, Denmark.

This study was partly funded by an unrestricted research grant from Allergifonden af 1981 and Jascha Fonden.

Received for publication in received form September 22, 1998.

Accepted for publication in revised form March 24, 1999.

MATERIALS AND METHODS

Patients

This study was undertaken according to the Helsinki declaration on human investigations and approved by the local ethics committee (protocol KF01-452/95). Informed consent was obtained from all subjects. Fifteen patients with HIV infections and clinical findings of eczematous skin manifestations, ie, papules, vesicles or honey-colored crusting, extensive serous weeping, folliculitis, pyoderma, and scaly erythematous dermatitis were included. The median age was 54 years (range 28 to 67 years). Concomitantly, eight patients were treated with antiretroviral medication (zidovudine, didanosine, saquinavir, and indinavir), and nine were treated with antifungal medication (ketoconazole and fluconazole).

Thirty milliliters of blood was drawn for histamine release experiments and IgE-immunoblotting. Skin prick test was performed the same day. Five healthy non-atopic age matched donors were included as negative controls.

Fungal Extracts

Fungal extracts included *Candida albicans*, *Penicillium notatum*, *Fusarium moniliforme*, and *Trichophyton rubrum*. Extracts were delivered lyophilized by Greer Laboratories, Lenoir, NC. The fungi had been cultivated in a liquid trypticase medium for 2 to 3 weeks before harvesting. The fungus mat was separated from the medium containing mainly extracellular components. Cellular and extracellular components were obtained by extracting mat (cellular extract) and medium (culture filtrate) in 0.01 M ammonium bicarbonate. For histamine release and skin prick test the fungal extracts were dissolved in 50% glycerol and kept at 4°C. *Pityrosporum ovale* was delivered in 50% glycerol by ALK-Abello, Hørsholm, Denmark. For IgE-immunoblotting the extracts were reconstituted with sterile water at the day of analysis.

Skin Prick Test

Skin prick tests with 1:10 wt/vol fungal extracts in 50% glycerol were performed according to EACCI Guidelines¹⁷ in duplicate. A 10 mg/mL histamine, 2 HCl solution was used as positive control and a 50% glycerol solution was used as negative control. Results were read after 15 minutes and transferred with a strip of tape to squared millimeter paper. The wheals were measured at the longest axis and perpendicular to it. The mean was calculated and a mean wheal reaction of 3 mm or more was considered positive since the mean wheal size in controls was less than 2 mm and the standard deviation was 0.2 mm.

Histamine Release Test

The leukocyte histamine release test was performed as previously described with glassmicrofibre-coated microtitre plates that selectively bind histamine.¹⁸ Briefly, 25 μ L of whole blood was incubated for 1 hour at 37°C with fungal extracts diluted with Pipes-AMC buffer consisting of 10 mM piperazine-N,N'-bis(2-ethanesulfonic acid), 140 mM sodiumacetate, 5 mM potassiumacetate, 30 mM Trizma-T-1503, 0.5 mM calcium chloride, and 1 mM magnesium chloride at pH 7.4. Aliquots were run in duplicate and tested at six 3.3-fold dilutions of 1:300 wt/vol fungal extracts. The histamine release was measured spectrophotofluorometrically with a Gilson fluorometer and expressed as nanogram histamine, 2 HCl per mL whole blood.¹⁸ Positive response was defined as a release greater than 45 ng histamine, 2 HCl pr mL blood.¹⁹

Sodium Dodecylsulfate-

Polyacrylamide Gel Electrophoresis

Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed with a modified method of Laemmli²⁰ using a 8% to 16% Tris-glycine Novex precast gel running in a XCell II Mini-Cell (EI 9001) (Novex, San Diego, CA, USA). The gels were loaded with 25 μ L fungal extracts (1 mg dry weight), boiled for 5 minutes with a reducing buffer containing 2- β -mercaptoethanol. Electrophoresis was

run for 90 minutes at 125V and protein bands were stained with Coomassie Brilliant Blue. The molecular weights were estimated using molecular-mass standards (4–250 kD, Novex).

IgE-Immunoblotting

Transfer of protein bands to nitrocellulose membranes (pore size 0.45 μ m) was performed with an electroblotter (Novex). After blotting, the membranes were washed for 1 hour at room temperature by rocking in TBS buffer (0.05 M Tris, 0.15 M NaCl, 0.005 M NaN₃, pH 7.4) containing 1% Tween 20. The membranes were incubated overnight at room temperature with patient sera diluted 1:10 in TBS buffer containing 0.1% Tween 20. Normal sera and human myeloma IgE (4000 U/mL) served as negative control samples. After washing, the strips were incubated for 2 hours at room temperature with 0.5 mL monoclonal goat anti-human IgE (diluted 1:1000) conjugated to alkaline phosphatase (Sigma) and the bands were visualized by addition of nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (Promega).

Total IgE

The Pharmacia CAP system using anti-IgE coated immunocaps were used to measure total IgE in the sera. The assay is calibrated according to the WHO serum standard (International Reference Preparation 75/502) and values are reported in kU/L. IgE values less than 150 kU/L were considered normal.

HIV Antibodies and CD4+ T

Lymphocytes

Antibodies to HIV-1 were detected in serum by using a commercial recombinant HIV-1/-2 enzyme immunoassay (Abbott, Chicago, IL) and were confirmed with an HIV-1 Western blot IgG assay (Diagnostic Biotechnology Ltd, Singapore, Thailand). Enumeration of CD4+ T lymphocytes was done by flow cytofluorometry.

RESULTS

Thirteen of the 15 patients (87%) responded to one or more of the fungi by

Table 1. Frequency Distribution of Positive Test Results from 15 HIV-Infected Patients

Allergen	Histamine Release	Skin Prick	IgE-Blotting
<i>C. albicans</i> cellular extract	11 (73%)	4 (27%)	6 (40%)
<i>C. albicans</i> culture filtrate	7 (47%)	3 (20%)	6 (40%)
<i>P. notatum</i> cellular extract	5 (33%)	4 (27%)	3 (20%)
<i>P. notatum</i> culture filtrate	2 (13%)	1 (7%)	1 (7%)
<i>F. moniliforme</i> cellular extract	1 (7%)	2 (13%)	2 (13%)
<i>F. moniliforme</i> culture filtrate	1 (7%)	3 (20%)	1 (7%)
<i>T. rubrum</i> cellular extract	2 (13%)	1 (7%)	1 (7%)
<i>P. ovale</i>	0	0	0
Patients responding to one or more fungi	13 (87%)	7 (47%)	7 (47%)

basophil histamine release and seven patients (47%) were positive by skin prick test and IgE-immunoblotting (Table 1). The IgE response was most often directed against *C. albicans*. Eleven of 15 patients (73%) responded to this yeast with histamine release. Respectively, five and seven patients were positive by the skin prick test and immunoblotting. Five patients (33%) reacted to *P. notatum* with histamine release whereas the other fungi generated less frequent responses. When assessed by the immunoblotting and skin testing *Penicillium notatum* was the

second most sensitizing fungus. The two different preparations of fungal extracts showed some variation in the test responses, eg, cellular extracts of *C. albicans* and *P. notatum* resulted in more frequent histamine release reactions and skin prick test responses than the culture filtrates. None of the control subjects released histamine to the fungal extract preparations and skin prick test or immunoblotting recorded no unspecific test results.

Candida albicans IgE-binding components were detected in 7 different patients (patients nos. 1, 2, 4, 5, 9, 10,

14) by immunoblotting analysis (Tables 2 a and b). The IgE-immunoblot clearly demonstrated different IgE-protein binding profiles of cellular extracts and culture filtrates. *C. albicans* showed 13 IgE-binding components in the cellular extract and 11 components in the culture filtrate. Four bands showed identical molecular weights of 30, 40, 46, and 55 kD. In contrast to the culture filtrate, the cellular extract also revealed IgE-binding components in the high molecular weight area (96 to 125 kD). The most frequently appearing bands were of molecular

Table 2a. IgE-Binding Components of *C. albicans* Cellular Extract

Pt. no.	125*	115	96	84	70	46-58	46	42	40	37	30	24	18
2	x	x	x			x		x			x	x	x
4					x						x		x
5			x	x			x	x			x		x
9			x					x	x		x		x
10								x	x	x	x		x
14								x			x		x
N = 6	1	1	3	1	1	1	1	5	2	1	6	1	6

* Molecular weight in kD.

Table 2b. IgE-Binding Components of *C. albicans* Culture Filtrate

Pt. no.	72*	55	46	44	40	33	30	23	19	17	12
1			x								
2	x	x	x	x	x	x	x	x		x	
5	x	x	x	x		x	x	x	x		x
9			x		x						
10			x		x						
14			x		x						
N = 6	2	2	6	2	4	2	2	2	1	1	1

* Molecular weight in kD.

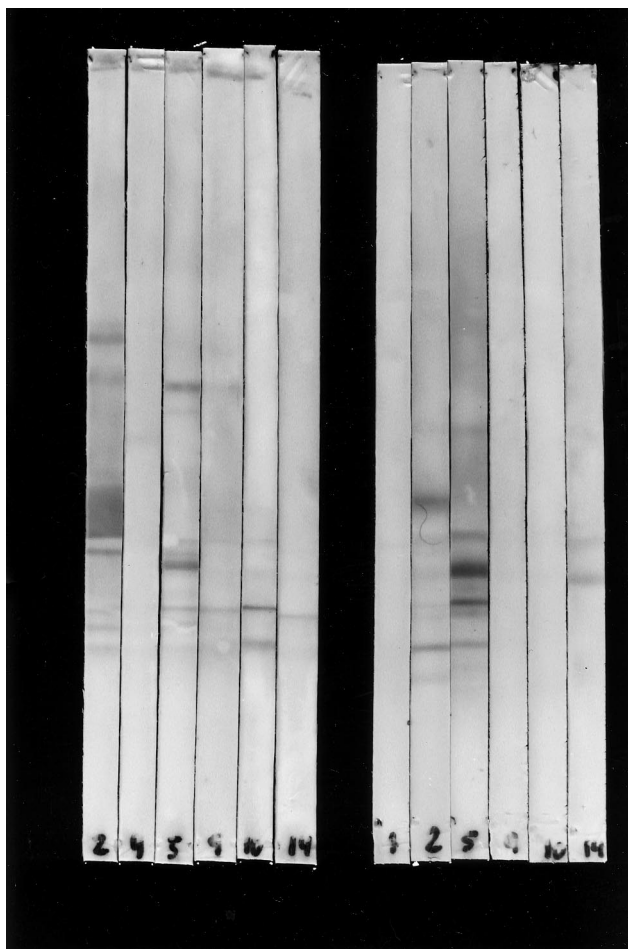


Figure 1. Immunoblot results of patients demonstrating IgE-binding to various components of *C. albicans*. From the left: *C. albicans* cellular extract (pt. 2, 4*, 5, 9, 10, and 14), *C. albicans* culture filtrate (pt. 1*, 2, 5, 9*, 10*, and 14). *These patients do not reproduce well on the photographs. Please refer to the tables. The molecular size of the components is shown in Table 2a-3b.

weights of 18, 30, and 42 kD in the cellular extract of *C. albicans* and 40 and 46 kD in the culture filtrate. Figure 1 shows representative immunoblot results of IgE-binding to various components of *C. albicans*. Sera from three patients revealed *P. notatum* IgE-binding components (Table 3). The blots showed 7 and 6 bands in the cellular and culture filtrates, respectively. Both preparations shared a 16-kD and 38-kD protein band, which bound IgE from one to three patients. Two patient sera displayed five IgE-binding bands with the *F. moniliforme* cellular extract and six bands with the culture filtrate and both blots showed a 32 and 56-kD component (data not shown). One pa-

tient reacted to *T. rubrum* and two IgE-binding components were identified, but none of the patients were found to respond to *P. ovale*. Figure 2 shows representative immunoblot results from patients demonstrating IgE-binding to various components of *F. moniliforme*, *P. notatum*, and *T. rubrum*. Immunoblotting with nonatopic sera and myeloma IgE did not demonstrate any unspecific IgE-binding. Seven patients (47%) demonstrated elevated IgE levels ranging from 491 to 30,744 kU/L with a mean IgE level of 3,021 kU/L and standard deviation of 7,840 U/mL. All 15 patients showed decreased CD4+ cell numbers, ie, less than 500 cells/ μ L (mean = 80/ μ L,

standard deviation 91). In 14 patients the CD4+ cell count was less than 200. Only in seven patients with elevated total IgE a correlation was found between IgE level and decrease in CD4+ count (correlation coefficient 0.51, $P < .02$ by Mann Whitney test). No association was found between IgE or CD4+ cell counts and the histamine release response, skin prick test, or immunoblotting.

DISCUSSION

Fungi may infect HIV-positive patients and some HIV-patients develop severe eczematous skin manifestations. All fungi included in this study have been reported to cause infections in HIV patients, especially *C. albicans*.¹⁶ Furthermore, the same fungi may play a role in IgE-mediated reactions in atopic patients.^{1,21,22} In a pilot study including 18 patients, we tested HIV-patients with and without eczema to the same panel of fungi. We found that predominantly patients with eczema responded with a positive test response (up to 60%, mean) to the fungi whereas a much smaller fraction of HIV patients without eczema showed positive test results (up to 23%, mean). We thus included the presence of skin manifestations in our selection criteria to test our hypothesis that IgE-mediated skin reactions to fungi may contribute to the skin reactions observed in some patients with HIV.

This study presents data indicating that selected fungi in HIV-infected patients may have allergenic significance. The majority of patients were sensitized to *C. albicans* and to *P. notatum*. A similar frequency of sensitization to *C. albicans* has been reported among patients with atopic dermatitis.⁹ *C. albicans* cross-reacts with *P. ovale*, however, we did not record any reactions to *P. ovale*.²³ This contrasts our previous findings among patients with atopic dermatitis where this yeast is an important allergen.⁹

Perhaps patients with HIV are less exposed to this yeast or the cross-reacting allergen is a minor allergen, which HIV-patients will not produce IgE antibodies against. The human IgE

Table 3a. IgE-Binding Components of *P. notatum* Cellular Extract

Pt. no.	52*	46	42	38	35	30	16
1		x	x	x	x	x	x
2	x						x
5	x						x
N = 3	2	1	1	1	1	1	3

* Molecular weight in kD.

Table 3b. IgE-Binding Components of *P. notatum* Culture Filtrate

Pt. no.	81*	38	32	28	24	16
1	x	x	x	x	x	x
N = 1	1	1	1	1	1	1

* Molecular weight in kD.

response appears to be directed against a relatively wide spectrum of fungal components of which *C. albicans* and *P. notatum* showed the largest variety of peptide bands. Both fungi also induced basophil histamine release in the majority of the sensitized patients. Previously, *C. albicans* has been shown to contain at least 42 different peptide bands⁹ and by using these extract preparations in the present study we detected 20 IgE-binding proteins. All patients revealed serum IgE directed against 18 and 30-kD components in the cellular extract of *C. albicans* and a 46-kD component in the culture filtrate. The 46-kD component may be identical to the 46-kD main allergen, previously identified as enolase.^{24,25} The histamine release test showed that most patients responded to the cellular extract of *C. albicans* whereas only half of the patients responded to the extracellular components in the culture filtrate. The two fungal extracts of *C. albicans* showed four bands with identical molecular weights. It is therefore likely that a complete separation of cellular and extracellular components was not obtained. Further purification and testing of the IgE-binding components should lead to a better extract for diagnosis.

For this reason, a pooling of cellular and culture prepared extracts charac-

terized by SDS-PAGE and IgE-blots may form the best basis for studies on the clinical relevance of *C. albicans* sensitization.

The discrepancy in the positive response rate of histamine release, skin

prick test, and immunoblot is not surprising. The methods detect IgE-sensitization by different methodologies, which identify allergenic epitopes through various immunologic mechanisms. The basophil histamine release test and skin prick test monitors the mediator release induced by IgE bound to the surface of basophils and skin mast cells, respectively, and the immunoblotting reveals unbound serum IgE directed against various fungal components. Furthermore, it is possible that the sodium dodecylsulfate-polyacrylamide gel electrophoresis may change the conformation, affinity, and epitope

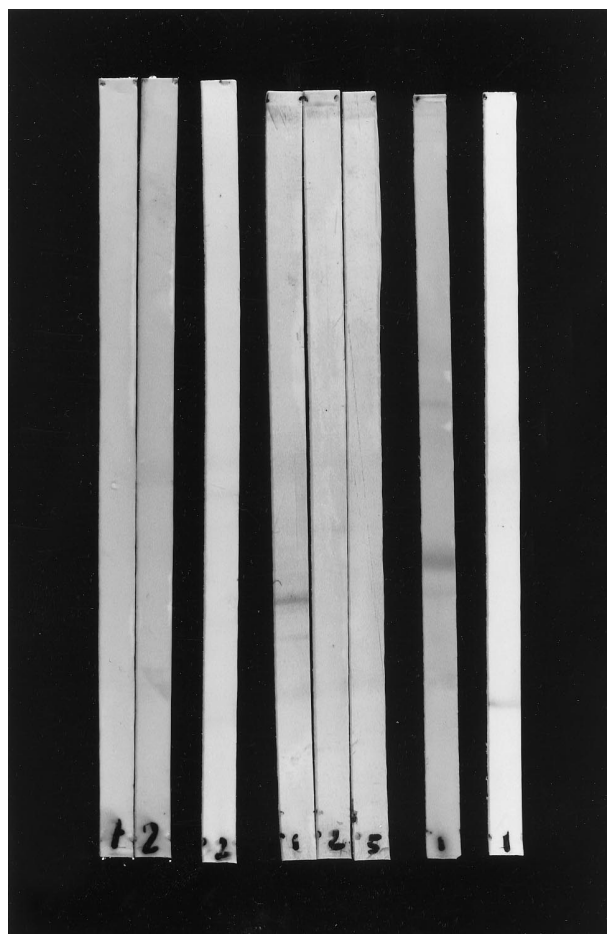


Figure 2. Immunoblot results of patients demonstrating IgE-binding to various components of *F. moniliforme*, *P. notatum*, and *T. rubrum*. From the left: *F. moniliforme* cellular extract (pt. 1*, 2*), *F. moniliforme* culture filtrate (pt. 2*), *P. notatum* cellular extract (pt. 1, 2*, 5*), *P. notatum* culture filtrate (pt. 1), and *T. rubrum* (pt. 1). *These patients do not reproduce well on the photographs. Please refer to the tables. The molecular size of the components is shown in Table 2a-3b.

density of the allergens. Another factor may be the allergen concentration applied. We do not know the major allergen content in mass units of these extracts and the concentration may have been too low for skin prick testing. Perhaps, the more sensitive intradermal skin test would have shown the same response rate as the basophil histamine release test. Nevertheless, skin and histamine release testing complements immunochemical methods by reflecting sensitization and functional importance of the allergenic extract tested. When comparing total serum IgE to CD4+ cell counts we were able to show an inverse correlation in the subgroup of seven patients with elevated IgE-level, but not in the entire group of HIV-patients. This is in agreement with studies similar to our, which were unable to show a significant correlation.^{26-28,30-32}

In conclusion, IgE-mediated reactions to a panel of fungal extracts were demonstrated by skin prick testing and basophil histamine release. *Candida albicans* was the most frequent inducer of IgE sensitization. This coincides with the clinical observation that the majority of HIV-infected patients have chronic or relapsing infections with this yeast compared to the other fungi. This suggests that these fungi are allergenic and may contribute to the inflammatory skin reactions observed in some patients infected with HIV and fungi.

REFERENCES

- Maggi E, Mazzetti M, Ravina A, et al. Increased production of IgE protein and IgE antibodies specific for fungal antigens in patients with the acquired immunodeficiency syndrome. *Res Clin Lab* 1989;19:45-49.
- Sample S, Chernoff DN, Lenahan GA, et al. Elevated serum concentrations of IgE antibodies to environmental antigens in HIV-seropositive male homosexuals. *J Allergy Clin Immunol* 1990; 86:876-880.
- Parkin JM, Eales LJ, Galazka AR, et al. Atopic manifestations in the acquired immune deficiency syndrome: response to recombinant interferon gamma. *Br Med J* 1987;294: 1185-1187.
- Dikeacou T, Löwenstein W, Romana C, et al. Relation of allergy to HIV infection. *J Eur Acad Dermatol Venerol* 1993;2:180-187.
- Goetz DW, Webb EL, Whisman BA, et al. Aeroallergen-specific IgE changes in individuals with rapid human immunodeficiency virus disease progression. *Ann Allergy Asthma Immunol* 1997;78:301-306.
- Koutsonikolis A, Nelson Jr RP, Fernandez-Caldas E, et al. Serum total and specific IgE levels in children infected with human immunodeficiency virus. *J Allergy Clin Immunol* 1996; 97:692-697.
- Israel-Biet D, Labrousse F, Tourani JM, et al. Elevation of IgE in HIV-infected subjects: a marker of poor prognosis. *J Allergy Clin Immunol* 1992;89:68-75.
- Magnan A, Vervloet D. AIDS: a model for the study of atopy. *Rev Mal Respir* 1995;12:177-183.
- Nissen D, Petersen LJ, Esch R, et al. IgE-sensitization to cellular and culture filtrates of fungal extracts in patients with atopic dermatitis. *Ann Allergy Asthma Immunol* 1998;81: 247-259.
- Ippolito F, Passi S, Caprilli F, et al. Seborrhea-like dermatitis in the acquired immunodeficiency syndrome. Clinical, histological, and microbiological aspects and biochemical findings. *Ital Dermatol Venerol* 1989;124:381-388.
- Groisser D, Bottone EJ, Lebwohl M. Association of *Pityrosporum orbiculare* (*Malassezia furfur*) with seborrheic dermatitis in patients with acquired immunodeficiency syndrome (AIDS). *J Am Acad Dermatol* 1989; 20:770-773.
- Ampel NM. Emerging disease issues and fungal pathogens associated with HIV infection. *Emerg Infect Dis* 1996; 2:109-116.
- Perfect JR, Schell WA. The new fungal opportunists are coming. *Clin Infect Dis* 1996;22:(suppl 2):112-118.
- Glasgow BJ, Engström RE Jr, Holland GN, et al. Bilateral endogenous *Fusarium* endophthalmitis associated with acquired immunodeficiency syndrome. *Arch Ophthalmol* 1996;114:873-877.
- Berger TG. Treatment of bacterial, fungal and parasitic infections in the HIV-infected host. *Semin Dermatol* 1993;12:296-300.
- Dronda F, Alonso-Sanz M, Laguna F, et al. Mixed oropharyngeal candidiasis due to *Candida albicans* and non-*albicans Candida* strains in HIV-infected patients. *Eur J Clin Microbiol Infect Dis* 1996;15:446-452.
- Malling HJ. Method of skin testing. *Allergy* 1993;48(Suppl):55-56.
- Nolte H, Schiøtz PO, Skov PS. A new microfibre-based histamine analysis for allergy testing in children. *Allergy* 1987;42:366-373.
- Nolte H, Heinig J, Hansen TK, Skov PS. Decision level analysis of leukocyte histamine release (LHR). *J Allergy Clin Immunol (News suppl)* 1994;2:2.
- Laemmli UK. Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature* 1970;227: 680-685.
- Steil W, Schultze-Werninghaus G, Staszewski S, et al. Increase of IgE-mediated sensitizations to mold spores and *Candida albicans* with increasing immunodeficiency in HIV-infected subjects, with relation to smoking habits. *Atemwegs Lungenkrankh* 1990;16: 371-373.
- Pedersen M, Permin H, Jensen C, et al. Histamine release from basophil leukocytes induced by microbial antigen preparations in patients with AIDS. *Allergy* 1987;42:291-297.
- Doekes G, van Ieperen-van Dijk AG. Allergens of *Pityrosporum ovale* and *Candida albicans*. I. Cross-reactivity of IgE-binding components. *Allergy* 1993;48:394-400.
- Savolainen J, Viander M, Einarsson R, et al. Immunoblotting analysis of concanavalin-A isolated allergens of *Candida albicans*. *Allergy* 1990;45:40-46.
- Kortekangas-Savolainen O, Kalimo K, Lammintausta K, et al. IgE-binding components of bakers yeast (*Saccharomyces cerevisiae*) recognized by immunoblotting analysis. Simultaneous IgE-binding to mannan and 46-48 kD allergens of *Saccharomyces cerevisiae* and *Candida albicans*. *Clin Exp Allergy* 1993;23:179-184.
- Ferrazzi M, De-Rinaldis ML, Salotti A, et al. Serum IgE levels in human immunodeficiency virus (HIV)-1 infected patients: correlation between

-
- IgE and CD4+ cells. Riv Eur Sci Med Pharmacol 1993;15:67-70.
27. Wright DN, Nelson RP Jr, Ledford DK, et al. Serum IgE and human immunodeficiency virus (HIV) infection. J Allergy Clin Immunol 1990;85:445-452.
28. Hovarth A. Total and specific IgE in sera of HIV positive and HIV negative homosexual male (regulation of IgE synthesis in HIV infection). Acta Biomed Ateneo Parmense 1992;63:133-145.
29. Fresard A, Vindimiam M, Genin C, et al. Immunoglobulins E and human immunodeficiency virus infection (HIV). Pathol Biol (Paris) 1994;42:750-753.
30. Miadonna A, Leggieri E, Tedeschi A, et al. Enhanced basophil releasability in subjects infected with human immunodeficiency virus. Clin Immunol Immunopathol 1990;54:237-246.
31. Ellaourie M, Rubinstein A, Rosenstreich DL. IgE levels in pediatric HIV-1 infection. Ann Allergy Asthma Immunol 1995;75:332-336.
32. Mazza DS, Grieco MH, Reddy MM, et al. Serum IgE in patients with human immunodeficiency infection. Ann Allergy Asthma Immunol 1995;74:411-414.
-