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Immunobiological Significance of Fungal and Bacterial Infections in Atopic Eczema

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ABSTRACT: Recurrent fungal and bacterial infections have a high frequency of association with atopic eczema (AE). A prospective study in our clinic in 110 AE patients and 30 healthy volunteers (age: 11 to 45 years) demonstrated a high colonization density of skin lesions, nasal, pharyngeal and vaginal mucosa with Staph. aureus in 102 cases, with streptococci in 53 cases and with Candida, Aspergillus or Penicillium sp. in 36 cases. Quantitative investigations of fecal and duodenal aspirate microflora in the same AE group revealed significantly increased counts of haemolytic coliforms, Candida/Geotrichum and pathogenic clostridia, generally associated with dramatically reduced counts of lactic acid producing bacteria. By contrast, positive skin cultures with Staph. aureus were isolated in only 2 controls and increased Candida counts in faeces were found in another 3 subjects. Specific IgE-antibodies (EIA) against Candida albicans, Aspergillus fumigatus and Saccharomyces cerevisiae were evident in 61, 32 and 56 cases, respectively, suggesting an increased infectious susceptibility and sensitivity to fungal antigens in the AE group. Thirty-one of 58 tested AE sera showed obviously decreased gammaglobulin levels (IgG and IgM, $p<0.005$) and in 24 of 35 patients tested for delayed cutaneous hypersensitivity reactions a severe depression of the cellular immune response was recorded. The different mechanisms responsible for the above findings are discussed. Our experience shows that correction of the intestinal and dermal dysbiosis along with appropriate nutritional support and immune modulating therapy are essential steps in the management of atopic eczema.

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Introduction

Associated opportunistic bacterial, viral and fungal infections are well-known hallmarks in the pathogenesis of Acquired Immunodeficiency Syndrome (AIDS)[1,2,3].

Atopic HIV-free patients show a similar pattern of superimposed dermal, mucosal and intestinal infections[4,5], suggesting a concomitant defect in the monocyte-macrophage system.

We have noticed that practically all investigated AE patients were carriers of chronic recurrent bacterial and yeast infections (especially with Candida sp. and Staph. aureus) mostly associated with a defective humoral and/or cellular immune function.

Our present report focuses on the immunobiological and clinical significance of yeast contamination in AE patients.

Patients and Methods

One hundred and ten clinically proved AE patients[6] (age: 11-45 years) and 30 healthy volunteers (age: 15-40 years) with no atopic history gave their consent to take part in this study.

All patients avoided any steroid or antihistaminic treatment for at least 10 days before admission and none had asthmatic symptoms. Seventy-one of them showed severe AE with widespread deep excoriation and weeping or bleeding lesions over the face, limbs and trunk; the remaining 39 patients were designated as having mild AE with erythema, xerosis, lichenification and superficial flexural excoriations. Intermittent diarrhoea, constipation, flatulence, intestinal rushes and abdominal discomfort (particularly after carbohydrate rich meals) were registered in 58 AE cases.

Neither microbial skin foci nor clinical signs of intestinal distress (excepting flatulence after meals in 6 cases) were recorded in the control group.

Microbiological Investigations: Oral, pharyngeal, nasal, vaginal and dermal microbiological samples were collected with sterile swabs using a standard technique.

Gastric and duodenal intubations were performed in 54 fasted patients in order to investigate possible contamination with pathogenic bacteria and yeast in the upper intestinal tract.

Anaerobically yielded fecal samples were taken and serial dilutions were plated for quantitative investigation of large bowel microflora.

All samples were cultured on appropriate growth-media for gram-positive, gram-negative, anaerobic and yeast strains.

Growth-media, incubation time, isolation, identification and quantitative estimation of microorganisms were described elsewhere[7,8]. Results are expressed in colony forming units / g wet stool.

Microbial strains showing colony numbers less than 1×10^4 /g feces for bacteria and less than 1×10^3 /g feces for fungi were not detectable with this method.

Immunological Tests: Total serum IgE and specific IgEs against three fungal antigens (Candida albicans, Saccharomyces cerevisiae and Aspergillus fumigatus) were measured in all 110 patients with standard enzyme-immunoassays (Padezym PRIST and Padezym RAST / German Pharmacia, FRG) whereby levels above 100 IU/ml for total IgE and RAST classes 2-4 were considered pathological.

Laser nephelometry techniques (Behring Werke, FRG) were used to evaluate serum IgG, IgM, IgA and total gammaglobulins in 58 AE patients and 21 controls using appropriate antisera (Behring Werke, FRG).

Cell-Mediated Immunity (CMI): The CMI function was evaluated in 35 AE patients (age: 16-30 years) and 17 healthy controls (age: 19-39 years) by means of a standardized multipuncture skin test for delayed cutaneous hypersensitivity (DCH) reactions (Multitest Merieux). Twenty-three of them had severe AE and the remaining 12 were mild.

The test device includes a glycerin control and 7 recall antigens standardized for DCH activity: tetanus toxoid, diphteria toxoid, streptococcus, tuberculin, Candida, tricophyton and proteus.

According to the instructions of the manufacturer (Institut Merieux, FRG) the 48 hr DCH reactions with a diameter of the induration larger than 2 mm were considered positive and Multitest-scores exceeding 5 mm for females and 10 mm for males were registered as normal.

The Student t-test was used to estimate the statistical significance of the results.

Results

1. Mucocutaneous Microflora

Our investigations demonstrate a high colonization density of the skin, oral, pharyngeal, nasal and vaginal mucosa of AE patients with Staph. aureus, enterococci, β-haemolytic streptococci and yeasts as Candida sp., Aspergillus and Penicillium sp. (Table 1).

By contrast, dermal Staph. aureus could be isolated in only 2 control subjects. Most AE patients showed mixed mucocutaneous bacterial and yeast infections especially with Staph. aureus, enterococci, Candida sp. and molds.

2. Intestinal Microflora

The quantitative estimation of duodenal aspirates and fecal microflora in AE patients (expressed in colony forming units/g wet stool or/

TABLE 1

Superimposed Infections of the Skin, Pharyngeal, Nasal and Vaginal Mucosa in 110 Atopic Eczema Patients and 30 Controls

	Haemolytic Staph. aureus	Enterococci and β-haemolytic streptococci	Candida albicans, Aspergillus sp., Penicillium sp.
AE patients			
n = 110	102	53	36
(p %)	(92,7%)	(48,2%)	(32,7%)
Controls			
n = 30	2	0	0
(p %)	(6,66%)	(0%)	(0%)

ml duodenal aspirate) consistently indicates significantly increased counts of haemolytic E. coli, Candida/Geotrichum sp., Proteus sp. and Clostridium perfringens, mostly associated with dramatically reduced numbers or absence of lactic acid producing bacteria in the large bowel (Table 2).

By contrast, fecal microflora in the control subjects revealed normal counts of lactobacilli ($>10^6$), bifidobacteria ($>10^8$), enterococci ($>10^6$). In all but three cases pathogenic strains of Candida, haemolytic coliforms or enterobacteriaceae were absent (Table 2).

Positive bacterial and yeast cultures were recorded in 21 duodenal fluid samples, mostly including Candida sp. and haemolytic coliforms. They showed a good correlation to the significantly increased numbers of yeasts and atypical E. coli in stools of atopic patients. On the other side atopic patients showing a drop in gram positive lactic acid-producing organisms associated with a sharp rise in fungal or atypic gram negative bacterial counts belonged to the severe eczema subset exhibiting multiple skin infections too.

3. Yeast Contamination and Antibody Response

Besides increased total IgE levels and frequent eosinophilia, specific IgE-antibodies against Candida albicans (61 cases), Aspergillus fumigatus (32 cases) and Saccharomyces cerevisiae (56 cases) antigens were evident in our AE patients.

TABLE 2

Fecal Microflora in 110 Atopic Eczema Patients and 30 Controls

	Lactobacilli	Bifido-bacteria	Haemolytic coliforms	Klebsiella	Proteus	Pathogenic clostridia	Candida/ Geotrichum
Normal range c.f.u./g wet stool	$> 10^6$	$>10^8$	$<10^4$	$<10^4$	$<10^4$	$<10^5$	$<10^3$
AE patients	Absent or $<10^4$	$<10^7$	$>10^6$	$>10^6$	$>10^5$	$>10^6$	10^4-10^7
n=110 (p%)	76 (69%)	31 (28,2%)	52 (47,3%)	36 (32,7%)	22 (20%)	40 (36,3%)	48 (43,6%)
Controls	2×10^4	$>10^8$	3×10^5	$<10^4$	$<10^4$	$<10^5$	$2{,}5\times10^4$
n=30 (p%)	3 (10%)	30 (100%)	2 (6,6%)	30 (100%)	30 (100%)	30 (100%)	3 (10%)

c.f.u. = colony forming units

Previous results from our laboratory[9] indicated that fungal antigens induce the highest frequency of specific IgE-antibodies, suggesting increased susceptibility to infection and sensitivity to yeasts in AE patients.

A surprisingly large subgroup of 31 of 58 AE patients showed lowered gammaglobulin levels (range 0.31-0.89 g/dl) associated in most cases with decreased serum total proteins. The difference was highly significant when compared to the control group ($p < 0.005$) and was mainly due to an obvious drop in the IgG and IgM values. By contrast, none of the control subjects showed specific IgE-antibodies or lowered gammaglobulin levels.

Twenty-six of 31 patients with lowered gammaglobulin and total proteins consistently showed raised counts of Candida/Geotrichum sp., atypical coliforms, Proteus and/or clostridia in stool samples associated with dramatically reduced counts of lactobacilli, bifidobacteria and/or enterococci.

4. Cellular Immune Response

A dramatic decrease of delayed cutaneous hypersensitivity (DCH) response was recorded in the AE group both in the male and the female subsets, whereby anergy was evident in 31,4 % and hypoergy in further 37.2% of the cases (Table 3).

Only one control subject was hypoergic and statistically there was a highly significant difference between the two groups ($p < 0,0001$).

No correlation could be established between Multitest-scores and total serum IgE levels. Twenty one (91.3%) AE patients with a lowered or absent DCH reaction belonged to the severe eczema subgroup, suggesting a direct relation between the defective CMI function and multiple recurrent bacterial and fungal infections.

The frequency of positive delayed responses for each antigen of the Multitest-system is summarized in Fig. 1. There was a significant decrease in delayed reactivity to all antigens in the AE group (particulary to Candida and Streptococcus sp.). Most positive results were registered in the mild eczema subset (Fig. 1).

Discussion

1. Mucocutaneous Microflora

In our experience persistence of chronic mucocutaneous infections in AE patients plays an important role in exacerbating the clinical course of the disease[4,10].

TABLE 3

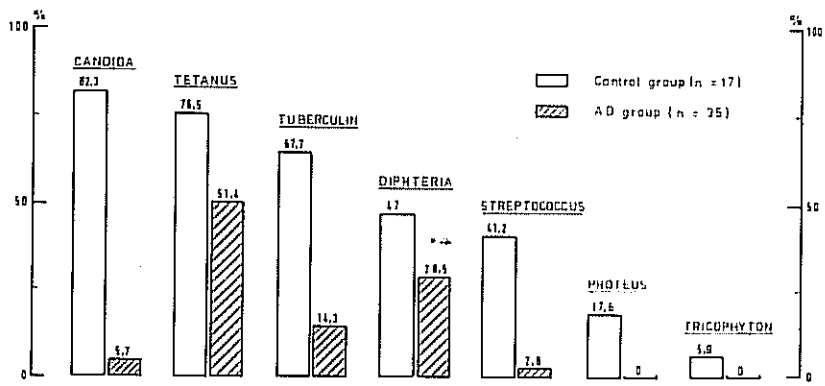
Delayed Cutaneous Hypersensitivity Response in AD Patients and Healthy Controls

	n	Skin reactivity			Multitest score (mm) Mean ± SD	Significance
		Normal	Hypoergy *	Anergy **		
AD patients ♀	23	10 (43.5%)	7 (30.3%)	6 (26.1%)	4.4 ± 3.5	$p < 0.0005$
Controls ♀	9	8 (88.9%)	1 (11.1%)	-	11.7 ± 2.2	
AD patients ♂	12	1 (8.3%)	6 (50.0%)	5 (41.7%)	2.8 ± 3.2	$p < 0.0001$
Controls ♂	8	8 (100%)	-	-	19.5 ± 4.4	
TOTAL ♀ + ♂						
AD patients	35	11 (31.4%)	13 (37.2%)	11 (31.4%)	3.9 ± 3.5	$p < 0.0001$
Controls	17	16 (94.1%)	1 (5.9%)	-	15.4 ± 5.2	

*Score < 5 mm for ♀ and < 10 mm for ♂
**Zero score

FIGURE 1

Frequency of Positive DCH Reactions for Each Antigen in Control Subjects and AD Patients.



The infectious agents activate the eczematous reactions by toxins acting directly on epidermal cells[11] or by allergic reactions against microbial antigens[12]. Most patients with widespread mucocutaneous infections belonged to the severe eczema subset.

Dermal and mucosal infections with Candida sp., Staph. aureus and other microbial pathogens are likewise common in HIV-infected patients and are thought to be of predictive value for the disease[13,14,15,16].

2. Intestinal Microflora

Although it was impossible to find a unique pattern of microorganisms in fecal samples from AE patients, the quantitative investigation of the most prevalent strains showed that the drop in gram positive, lactic acid-producing bacteria, was generally associated with an obvious rise in clostridia, fungi and/or pathogenic gram negative forms (Table 2).

The described intestinal bacterial and fungal overgrowth in the atopic group seems to be responsible for significant absorption and permeability changes in these patients[4,8,9]. Further important biologic side-effects of the intestinal overgrowth syndrome in AE (increased indicanuria, lactose malabsorption, increased fecal fat excretion, lowered total serum proteins) are reported elsewhere[8].

Enteric infections associated with chronic diarrhoea and significant weight loss frequently cause morbidity and mortality in AIDS patients[16]. Alterations in mucosal immunity explain the increased incidence of enteric infections in atopic[4,8] psoriatic[15] and HIV[17] subjects. The accurate identification of pathogenic strains along with appropriate "in vitro" resistance tests supply valuable information for the right topical, intestinal or systemic antimicrobial therapy in these cases.

3. Yeast Contamination and Antibody Response

The markedly diminished gammaglobulin levels in over 50% of the tested AE sera may be due either to an inherited defect or to an acquired deficiency following an increased immunglobulin turn-over and/or consumption in circulating immune complexes with food or microbial antigens[9].

It therefore seems likely that the deficient humoral immune status may be related to an increased cutaneous and/or intestinal germ contamination[8,10,20] causing skin/mucosal damage and abnormal antigen entry with resulting sensitisation[21]. In turn, a non-specific suppression or the humoral immune response following systemic infection by Candida albicans in experimental animals has also been reported[22,23].

4. Cellular immune response

To our knowledge several mechanisms may account for the impaired cellular immune response in AE patients:

I. A defective neutrophil chemotaxis due to increased histamine concentrations[24].
II. Circulating immune complexes might inhibit chemotaxis of phagocyting neutrophils[25] and the antibody dependent cell-mediated cytotoxicity of T and "null" lymphocytes[26].
Increased histamine and circulating immune complex levels as well as a markedly reduced lymphocyte cytotoxic function have already been reported in AE patients[9,27].
III. The generation of phospholipase A_2 by Candida[28] triggers increased phospholipid degradation with activation of the arachidonic acid pathway and increased production of pro-inflammatory prostaglandins and leukotrienes.
PGEs and LTB_4, in turn, may inhibit lymphocyte

proliferation, generation of lymphokines and T-lymphocyte-mediated cytotoxicity[29,30]. Zinc, gamma-linolenic and dihomo-gamma-linolenic acid supplementation are known to improve these conditions[31,32].

IV. The synergistic effect on animal mortality in combined infections with Candida albicans and Staph. aureus[33] as well as suppression of humoral and cellular immune responses following inoculation with Candida albicans was demonstrated in other studies[22,23]. A direct non-specific suppressive effect of mold toxins upon macrophage migration has also been reported in earlier experiments[34,35].

We believe that chronic yeast infections in atopic, psoriatic or AIDS patients may be promoted by primary or secondary deficiencies of the host immunity and that, in turn, fungal and bacterial agents may alter significantly the humoral and cellular immune function in these patients.

As defects of the T-cell function are known to be different in AIDS and in AE patients, the above data suggest alterations in the macrophage-monocyte function leading to a severe increase in infectious susceptibility.

Correction of intestinal and dermal dysbiosis along with appropriate diet and immune modulating therapy are, according to our experience, essential steps in the management of atopic eczema.

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