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Persistent Generalized Lymphadenopathy: Immunological and Mycological Investigations

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Immunological and mycological investigations were carried out in 21 Swedish homosexual males. One of them had AIDS, one pre-AIDS and 19 lymphadenopathy of whom 18 fulfilled the criteria of persistent generalized lymphadenopathy (PGL) as defined by the Centers for Disease Control, Atlanta, (CDC). The patients were investigated immunologically with respect to their in vitro lymphocyte reactivity to various mitogens. The patients with AIDS and pre-AIDS belonged to the group of 8 patients with low response to mitogens. Blood helper T cell percentages and serum β_2-microglobulin concentrations correlated with the PHA reactivity. Three patients, with the diagnoses AIDS, pre-AIDS and PGL respectively, had clinical signs of oral candidiasis with rich growth of Candida albicans in culture. These were all low responders to mitogen stimulation. Six cases of tinea pedis were diagnosed and seemed to be distributed among the patients irrespectively of the severity of their immunological disorders. *Key words: AIDS; Homosexual; Beta-2-microglobulin; T-lymphocyte; Lymphocyte transformation; Candidiasis; Dermatophytosis.*
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Persistent generalized lymphadenopathy (PGL) in homosexual males constitutes a growing and serious medical problem. For epidemiological purposes PGL has been defined by the Centers for Disease Control (CDC) in Atlanta, USA, as lymphadenopathy with lymph node diameter of at least 1 cm involving two or more extrainguinal sites for more than three months in the absence of any current illness or drug use known to cause lymphadenopathy. This syndrome is epidemiologically and aetiologically related to the acquired immune deficiency syndrome (AIDS). PGL has been shown to precede the development of AIDS in homosexuals. LAV/HTLV-III virus and antibodies against that retrovirus have been shown in a high frequency of patients suffering from AIDS and PGL, respectively (1, 2). Immunological investigations in this group of patients have demonstrated various immunological disorders indicating different grades of immune deficiency (3, 4, 5, 6), the most characteristic feature being depletion of cells with T helper/inducer phenotype. Proliferative responses to mitogens have been reported to be more or less affected.

In this paper we present results of an immunological and mycological investigation in a group of Swedish patients with AIDS or PGL. Proliferative responses of lymphocytes to various mitogens were related to percentages of blood T helper cells and serum levels of β_2-microglobulin. The patients were examined for clinical signs of oral candidiasis and cultures were made from the oral cavity. Also the prevalence of dermatophyte infections was investigated.

Table I. *Diagnoses, serum β_2-microglobulin concentrations and blood T helper cells*

β_2-Microglobulin upper normal limit (mean + 2 SD): 2.5 μg/ml. T helper cells (anti-Leu 3a) normal frequency: 45±10%. PGL = persistent generalized lymphadenopathy

Patient number	Diagnoses	β_2-microglobulin (μg/ml)	T helper (%)
1	PGL	–	–
2	PGL	2.5	55
3	PGL	2.4	39
4	PGL	3.7	27
5	PGL	–	33
6	PGL	2.1	–
7	PGL	–	–
8	PGL	2.4	–
9	pre-AIDS	3.1	17
10	PGL	2.3	55
11	PGL	1.5	46
12	PGL	2.2	80
13	AIDS	4.4	<0.5
14	PGL	2.8	–
15	PGL	3.7	24
16	PGL	–	–
17	PGL	2.2	–
18	PGL	–	35
19	PGL	–	32
20	PGL	3.3	–
21	Lymphadenopathy[a]	2.3	40

[a] The CDC criteria of PGL not fulfilled.

MATERIAL AND METHODS

Subjects. 21 homosexual males were examined. The median age was 32 years (range 23 to 49). Table I shows the diagnoses, where pre-AIDS is a clinical condition closely related to AIDS.

Separation of lymphocytes. Heparinized venous blood was centrifuged on Ficoll-Hypaque.

Lymphocyte stimulation. The proliferative responses to Phytohaemagglutinin (PHA), Concanavalin A (ConA) and Pokeweed mitogen (PWM) were studied by means of determination of DNA synthesis. PHA was used at concentrations of 0.25, 2.5 and 25 μg/ml, ConA at 2, 10 and 50 μg/ml and PWM at 0.05, 0.5 and 5 μg/ml. Triplicate cultures were established in 96-well microtiter plates with 2×10^5 cells per well. Cell cultures were incubated for three days. The mitogen induced DNA synthesis was assayed by labelling cells with 0.20 μCi/ml of ^{14}C-thymidine during the final 18 h of incubation. The incorporated radioactivity was measured in a liquid scintillation counter. Tests were parallelly carried out with lymphocytes from apparently healthy sex- and age-matched controls.

Immunofluorescence test for phenotypic markers. The lymphocyte subset reacting with fluorescein isothiocyanate (FITC-) conjugated monoclonal antibodies (Becton Dickinson) directed against helper/inducer T cells (anti-Leu 3a) was determined by immunofluorescence as described earlier (7).

β_2-microglobulin. Serum levels of β_2-microglobulin were determined by a competitive radioimmunoassay (Pharmacia Phadebas®).

Mycological examinations. The patients were examined for clinical signs of oral candidiasis and cultures were made to show the colonization of yeasts in the oral cavity. Specimens were collected from the oral mucosa with a swab which was immediately plated on Sabouraud's glucose agar. The plates were incubated at room temperature for two weeks and isolated yeasts were identified according to standard criteria (8). Yeast colonies were counted and growth classified as rich (>50 colonies), moderate (6–50 colonies) or sparse (1–5 colonies).

Skin scrapings were collected from the toe webs of all the patients irrespectively of any symptoms. Furthermore, specimens were collected from other sites, if signs of dermatophytosis were noted. The samples were inoculated on Sabouraud's glucose agar and Dermatophyte test medium (DTM) and

Table II. *Lymphocyte reactivity to ConA, PHA and PWM*

Results in per cent of the mean response of the controls

Patient number	ConA 2 μg/ml	PHA 0.25 μg/ml	PWM 0.05 μg/ml
1	128.1	184.2	132.6
2	149.1	56.7	189.4
3	125.3	130.9	154.4
4	36.3	21.0	19.7
5	31.0	119.3	9.1
6	125.6	169.1	133.9
7	20.7	39.3	31.4
8	59.4	68.7	17.0
9	0	4.5	0
10	69.0	173.9	5.8
11	75.0	147.6	36.3
12	76.0	141.9	30.5
13	4.6	1.0	2.4
14	97.4	155.4	129.9
15	64.7	26.3	46.7
16	51.0	71.2	0
17	144.5	100.6	193.4
18	175.0	2.5	213.2
19	50.8	33.5	118.7
20	0	64.3	0
21	29.6	0	0

cultured for four weeks at room temperature. Dermatophytes were identified according to their colony morpholoy and microscopic appearance, using standard criteria (9).

RESULTS

The lymphocyte responses to PHA, ConA and PWM are shown in Table II. The three concentrations of each mitogen yielded fairly equal results. The reactivities to suboptimal concentrations of mitogens, however, seemed to be the most sensitive to discriminate between normal and abnormal responses and are therefore presented. The results are given in per cent of the mean response of the 17 controls. Reduced lymphocyte responses were seen for the patients numbered 4, 7, 9, 13, 15, 16, 20 and 21. Of these patients one was suffering from AIDS and one from pre-AIDS. The reactivities of these low responders to PHA are shown in Fig. 1.

The percentages of blood T helper cells and serum β_2-microglobulin concentrations are shown in Table I for patients examined within two months from the lymphocyte stimulation test. T helper percentages correlated with the lymphocyte response to PHA ($p<0.05$, $r=0.66$) (Fig. 2). β_2-microglobulin concentrations, correlated inversely with PHA reactivity ($p<0.01$, $r=-0.67$), (Fig. 2).

Candida albicans was recovered from the oral mucosa of 17 patients (81%) (Table III). No additional yeasts were seen. Rich growth of Candida albicans was found in 6 patients (29%). Evidence of clinical infection with rich growth of Candida albicans in culture was demonstrated in three patients (numbers 9, 13 and 20). Three clinical variants were seen: red atrophic lesions of the buccal mucosa, white to grey pseudomembranous leisons and leukoplakia of the tongue. Epithelial smears were obtained from the two patients with red

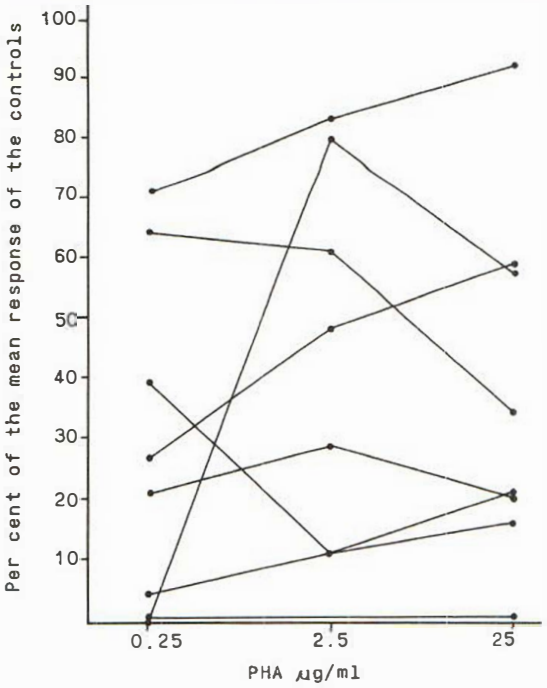

Fig. 1. Lymphocyte reactivity to PHA in 8 patients with reduced mitogenic responses.

atrophic patches and leukoplakia respectively. Mycelial forms of yeast were present in both smears.

Dermatophytes were isolated from the feet, usually toe webs, of 6 patients (29%) (Table III), all with clinical signs of tinea pedis. The usual dermatophyte population was represented. No dermatophytes were isolated from other sites in any patient.

DISCUSSION

Studies performed in USA and France (5, 4) have demonstrated that mitogen responsiveness may be reduced in patients with AIDS and PGL. Our patients with AIDS and pre-

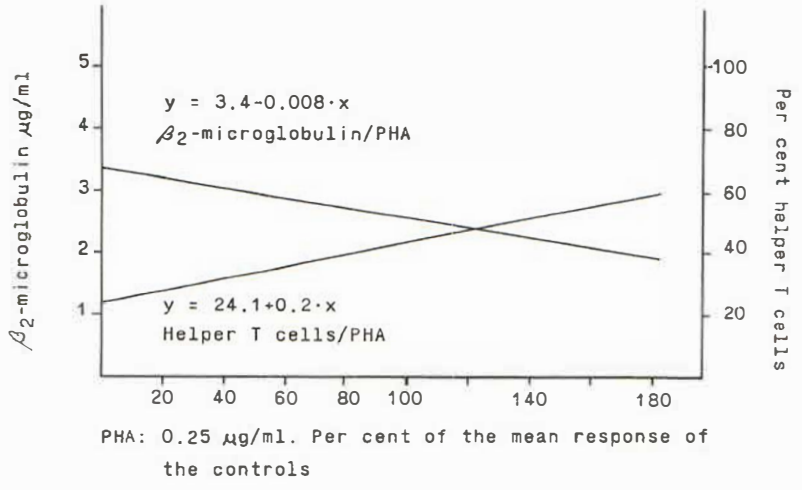

Fig. 2. Relation between PHA reactivity and frequencies of blood helper T cells and serum β_2-microglobulin concentrations respectively.

Table III. *Candida albicans recovered in culture from the oral cavity and dermatophytes isolated from the feet*

+++ = rich growth, ++ = moderate growth, + = sparse growth, − = no growth

Patient number	Yeast culture	Dermatophyte culture
1	−	Trichophyton rubrum
2	−	−
3	+	Trichophyton rubrum
4	+++	Trichophyton rubrum
5	++	−
6	++	−
7	+	Trichophyton mentagrophytes
8	++	−
9	+++	−
10	+	−
11	+	Epidermophyton floccosum
12	++	−
13	+++	−
14	−	−
15	+++	−
16	++	−
17	++	−
18	−	−
19	++	−
20	+++	−
21	+++	Trichophyton mentagrophytes

AIDS were low responders. In contrast to this, only five of 18 patients with PGL had clearly reduced mitogen responses. One patient with persistent lymphadenopathy did not fulfil all the criteria of PGL but belonged to the group of low responders to mitogens. The results indicate that our patients with PGL have varying degrees of immune deficiency, presumably reflecting the severity of the disease or different phases of the disease.

β_2-Microglobulin is a low molecular weight protein that is found on the surface of nucleated cells. It is released into the blood as a result of cell turnover. It has been shown previously that β_2-microglobulin concentrations correlate inversely with blood T helper cell counts in homosexual men (10). The percentages of T helper cells and the concentrations of β_2-microglobulin in our patients were both correlated with the PHA responsiveness (Fig. 2), which was to be expected as it is known that PHA stimulates mainly T cells of helper subset.

Candida albicans is a normal inhabitant of the human alimentary tract. However, previous studies of oral candida colonization (11, 12) have not reported prevalence figures of the magnitude seen in our patients (81%). Oral candidiasis may be the result of a diversity of conditions but it is particularly prevalent in patients with severe immune defects. Three of our 8 patients with reduced mitogen responsiveness had a clinically manifest candidiasis with rich growth of Candida albicans in culture from the oral cavity. Additional three of these 8 patients showed rich growth of Candida albicans without clinical infection. Thus oral candidiasis seems to correlate well with decreased mitogen reactivity in this group of patients.

The dermatophytoses are among the most common infections in man and tinea pedis is by far the most common fugus disease. The reason why some people contract the disease

is unknown but cellular immune reactivity has been reported to be of great importance for the antifungal resistance of the host (13). For this reason it was of interest to investigate the prevalence of tinea pedis in our patients. Three of 8 patients (38%) with reduced lymphocyte reactivity to mitogens had a tinea pedis compared to three of 13 patients (23%) in the group with essentially normal lymphocyte responses. However, none of the two patients with AIDS or pre-AIDS had clinical or mycological signs of dermatophytosis. Extended investigations are in progress to evaluate the prevalence of dermatophytosis in homosexual males with varying degrees of immune deficiency.

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