

IMMUNOLOGICAL ABNORMALITIES OF ACQUIRED IMMUNODEFICIENCY SYNDROME AND RELATED DISORDERS IN PATIENTS FROM RIO DE JANEIRO, BRAZIL

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The immunological profile of acquired immunodeficiency syndrome (AIDS) and chronic lymphadenopathy syndrome (CLAS) in 15 and 11 Brazilian patients, respectively, was studied. The AIDS patients showed reduced percentage of total T (CD3) and T-helper-inducer (CD4) lymphocytes, relative increase in numbers of T-suppressor-cytotoxic (CD8) cells and a marked inversion of T-helper-inducer/suppressor-cytotoxic (CD4/CD8) ratio. Lymphoproliferative responses to PHA, ConA, PPD and PWM were diminished. Hypergammaglobulinemia and high levels of circulating immune complexes were also found. The CLAS patients also showed important immunological alterations, but not so intense as those with AIDS. These data seems to be similar to those observed in other parts of the world.

Key words: AIDS – lymphocyte and humoral alterations

More than 47000 cases of acquired immunodeficiency syndrome (AIDS) have been reported in the United States since September 1981 (W. H. O., 1987). At that time AIDS was recognized as a new disease, consisting of opportunistic infections and/or the development of neoplasms, especially Kaposi's sarcoma and marked abnormalities of immunological functions (Fauci et al., 1985; Bowen et al., 1986). Since then, cases of AIDS have been reported in about 40 countries in all continents constituting a pandemic (W. H. O., 1987). The etiologic agent of AIDS, the human immunodeficiency virus (HIV) also known as human T-cell lymphotropic virus type III (HTLV-III) (Popovic et al., 1984) or AIDS-related virus (ARV) (Levy et al., 1985) was first isolated as lymphadenopathy associated virus (LAV) in 1983 (Barré-Sinoussi et al., 1983). Up to November 1987, 2237 cases of AIDS have been described in Brazil since 1982, with the majority of cases concentrated in the two major cities of São Paulo and Rio de Janeiro (Ministério da Saúde, 1987). Although the immunological profile of AIDS has been well characterized in other nations (Piot et al., 1984; Bowen et al., 1986), only clinical and epidemiological aspects

of the disease have been studied in this country so far (Ministério da Saúde, 1987; Galvão-Castro et al., 1987). The immunologic profile of some patients with AIDS in Rio de Janeiro is described in this paper.

MATERIALS AND METHODS

Patients: twenty six patients, divided into two groups, were studied: a chronic lymphadenopathy syndrome (CLAS) group, consisting of 11 individuals (1 female and 10 males), with the ages ranging from 22 to 45 years (mean age 31.8); and a full-blown acquired immunodeficiency syndrome (AIDS) group, consisting of 15 subjects (2 females and 13 males), with the age ranging from 15 to 48 years (mean age 32.3). The patients were clinically defined according to the criteria of the Centers for Diseases Control, USA (CDC, 1982).

The control groups included 27 healthy heterosexual (HHT) individuals who were matched with the patients for age and sex; and 10 healthy homosexual males (HHM), who were matched with the patients for age, colour and risk groups (Table).

All of the patients but 2 of the AIDS group were HIV seropositive in indirect immunofluorescence on HIV-infected H9 cells, kindly provided by Dr. Robert Gallo (Laboratory of

These studies were supported by the Ministry of Health, Brazil.

Received December 23, 1987.

Accepted March 17, 1988.

TABLE
Groups studied, age, colour and immunoglobulin levels

Groups studied	Age	Colour*		Immunoglobulins (mg/dl)		
	(years)	Wh	Mt	IgG	IgM	IgA
<i>Controls:</i>	**			**	**	**
Healthy heterosexuals	33.0 ± 11.3	10	1	1767 ± 470	240 ± 80	280 ± 123
Healthy homosexual males	33.1 ± 10.3	10	0	1598 ± 717	219 ± 117	263 ± 134
<i>Acquired immunodeficiency syndrome:</i>	32.3 ± 9.9	13	2	2188 ± 842	371 ± 203	480 ± 288
Homosexual males***	32.8 ± 8.9	7	2	2344 ± 817	355 ± 183	594 ± 275
Bisexual males	35.0 ± 11.5	4	0	2011 ± 214	254 ± 54	328 ± 252
Blood transfusion ⁺	15.0	1	0	1560	840	215
Bisexual's partner ^{+/++}	34.0	1	0	1940	400	175
<i>Chronic lymphadenopathy syndrome:</i>	31.5 ± 8.5	10	1	1929 ± 764	279 ± 175	279 ± 120
Homosexual males	31.2 ± 7.7	4	1	1889 ± 719	218 ± 82	278 ± 65
Bisexual males	24.2 ± 8.3	5	0	1644 ± 419	242 ± 52	221 ± 80
Bisexual's partner ⁺	45	1	0	3560	775	570

* Wh = white, Mt = mulatto; ** all values are mean ± 1 SD; *** one had negative serology; ⁺ females; ⁺⁺ negative serology.

Tumor Cell Biology, National Cancer Institute, Bethesda, Maryland, USA) using the method described by Mortimer et al. (1985), enzyme-linked immunosorbent assay (the ELAVIA from Institute Pasteur, Paris – France) performed as recommended by the manufacturers and a Dot-enzyme immunoassay using a method described by Ivo-dos-Santos et al. (1987). These two AIDS patients were also seronegative on a Western blot (WB) test (Schupbach et al., 1984). All the control individuals, with the exception of one healthy homosexual male, were HIV seronegative.

Blood cells and plasma collection: a total of 25 ml of peripheral venous blood was collected in a sterile tube containing ethylene diamine-tetraacetic acid (EDTA), 30 mM final concentration. The mononuclear cells were prepared by means of density centrifugation with Histopaque-1077 (Sigma Diagnostics, St. Louis, USA). Plasma was collected and stored at -70°C up to 3 months.

T-lymphocyte quantification: the murine monoclonal antibodies OKT3, OKT4 and OKT8 (Ortho Diagnostic Systems, New Jersey, USA) were used, respectively, to quantitate the total T-lymphocytes, T-helper/inducer and T-suppressor/cytotoxic (sub)sets, by indirect immunofluorescence (IIF) using fluorescent anti-mouse IgG, as described by Kung et al. (1979) and Piot et al. (1984). The results were

expressed in percentages of the total mononuclear cells.

Lymphoproliferative responses (LR): the LR procedure was performed as described by Oppenheim & Schechter (1980). Mitogens used were phytohemagglutinin (PHA) (5µg/ml; Sigma, Chemical Company, St. Louis, USA), concanavalin A (Con-A) (10µg/ml; Sigma, Chemical Company, St. Louis, USA), purified protein derivative (PPD) (50µg/ml; from BCG supernatant, Moreau strain, kindly provided by Prof. A. Oliveira Lima, Fundação Ataulfo de Paiva, Rio de Janeiro, Brazil) and pokeweed mitogen (PWM) (5µg/ml; Sigma, Chemical Company, St. Louis, USA).

Immunoglobulin levels: plasma levels of IgA, IgM and IgG were determined by single radial immunodiffusion (Mancini et al., 1965) using Nor-Partigen immunodiffusion plates (Behring Institute, Marburg, Germany). Results were expressed in mg/dl.

Immune complex quantification: the method used was the 125I-C1q-binding assay as described by Zubler et al. (1976).

Statistical analysis: results were analysed statistically according to the Kruskal-Wallis test, the U-Mann-Whitney's test and the Spearman's correlation test.

RESULTS

Circulating T lymphocytes: the percentage of circulating T lymphocytes was significantly reduced in 9 out of 15 patients with AIDS compared to the healthy heterosexual group ($p < 0.001$). All of them showed diminished percentage of T-helper lymphocytes as compared to the healthy heterosexuals ($p < 0.001$) or to the healthy homosexual males ($p < 0.01$; Fig 1a). The CD4/CD8 lymphocyte ratio was markedly diminished in the patients with AIDS as compared to the healthy heterosexuals ($p < 0.001$) or the healthy homosexual male group ($p < 0.001$; Fig. 1b).

Patients with CLAS showed a significantly diminished percentage of both T-helper cells and CD4/CD8 cell ratio as compared to the healthy heterosexuals ($p < 0.001$ and $p < 0.001$) and to the healthy homosexual males ($p < 0.05$ and $p < 0.05$), respectively. All but 2 (81.9%) had T-helper lymphocyte percentages below the normal range and 7 out of 11 displayed a decreased CD4/CD8 cell ratio (Figs. 1a and 1b).

A significant reduction (less than the 90th percentile of the heterosexual group) in the percentage of both T-helper lymphocytes and CD4/CD8 cell ratio was observed, respectively, in 4 and 3 out of the 10 individuals of the healthy homosexual male group (Figs. 1a and 1b).

Lymphoproliferative responses: As shown in Fig. 2 peripheral blood lymphocytes from AIDS patients had a reduced ability to respond to Con-A (10 out of 10, $p < 0.001$); PHA (9 out of 10, $p < 0.001$); PPD (4 out of 7, $p < 0.001$) and PWM (3 out 7, $p < 0.001$) as compared to the healthy heterosexual group (Fig. 2). The CLAS patients presented a diminished response to Con-A (5 out of 7, $p < 0.001$) and PHA (5 out 7, $p < 0.01$) as compared to the healthy heterosexual group. However, no statistically significant differences were noted between the CLAS and the healthy homosexual male groups for these two mitogens. On the other hand, statistically significant difference in the stimulation with PHA ($p < 0.01$) was observed when comparing the healthy homosexual males to the healthy heterosexual group.

Circulating immune complex (CIC) and immunoglobulin levels: there were increased Ciq-binding activities (Ciq-BA) over the 90th percentile in 91.6% of the AIDS patients with a mean value of 35.4% (Fig. 3). The levels were significantly different from those observed in the healthy heterosexual, healthy homosexual male group and CLAS groups ($p < 0.001$).

In 40% of the CLAS patients, the Clq-BA were abnormally higher ($p < 0.05$) than in the healthy heterosexual group, with a mean value of 13.7%, but these patients did not differ significantly from the healthy homosexual male group.

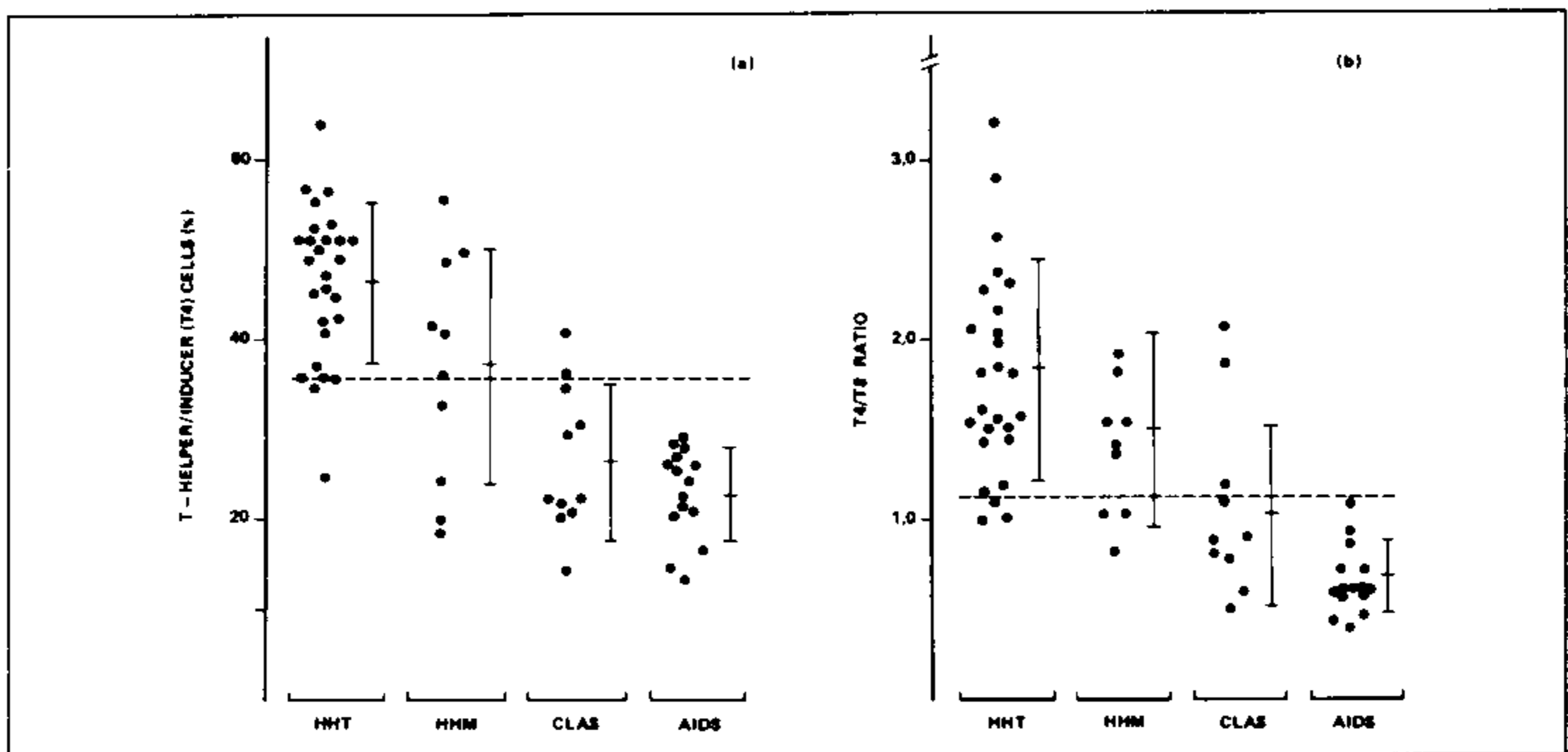


Fig. 1: (a) T-helper/inducer (T4) cells; (b) T4/T8 ratio. Values above the discontinued lines represent the normal range (90th percentile of the healthy heterosexual group). Vertical bars represent the mean \pm 1 standard deviation of the different groups. HHT, healthy heterosexuals; HHM healthy homosexual males; CLAS, chronic lymphadenopathy syndrome; AIDS, acquired immunodeficiency syndrome.

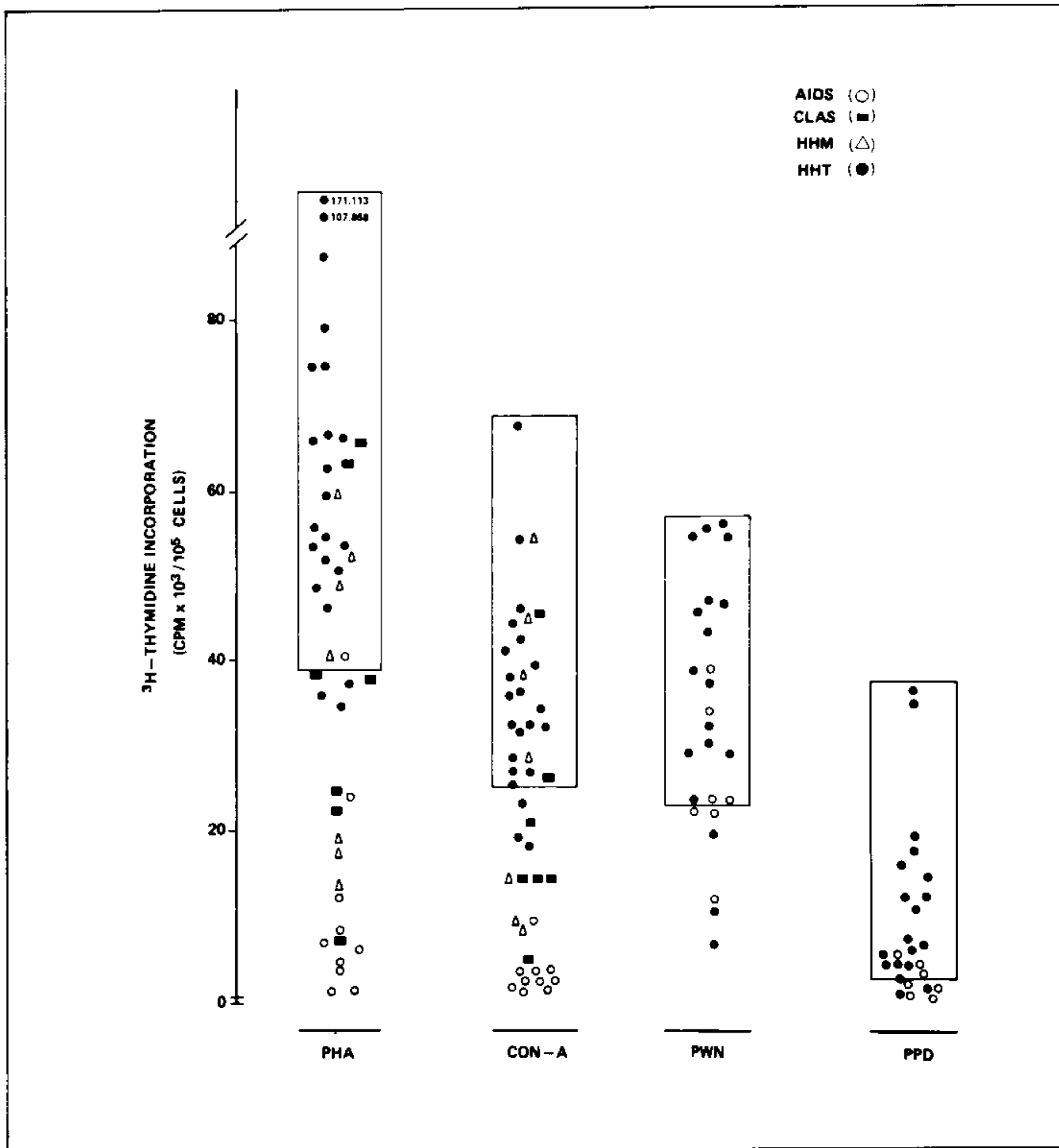


Fig. 2: lymphoproliferative responses to PHA, Con-A, PWM and PPD. The normal range (90th percentile) in the HHT group is indicated by the rectangular areas.

DISCUSSION

Both AIDS and CLAS groups displayed hypergammaglobulinemia. The AIDS group, when compared to the healthy homosexual males, showed significant differences for the three isotypes studied ($p < 0.05$). However, IgA was the only significantly elevated class in the AIDS group when compared with the healthy heterosexual group ($p < 0.05$; Table). No correlations were found between CIC and immunoglobulin levels.

Our results showed that Brazilian AIDS patients displayed abnormalities of the immune system similar to those already described in other countries (Piot et al., 1984; Fauci et al., 1985; Bowen et al., 1986; Quinn et al., 1987). Indeed, it was observed selective CD4 cell depletion, which resulted in the CD4/CD8 ratio inversion, and diminished T-lymphocyte response to mitogens to soluble PPD antigen in patients with AIDS and CLAS.

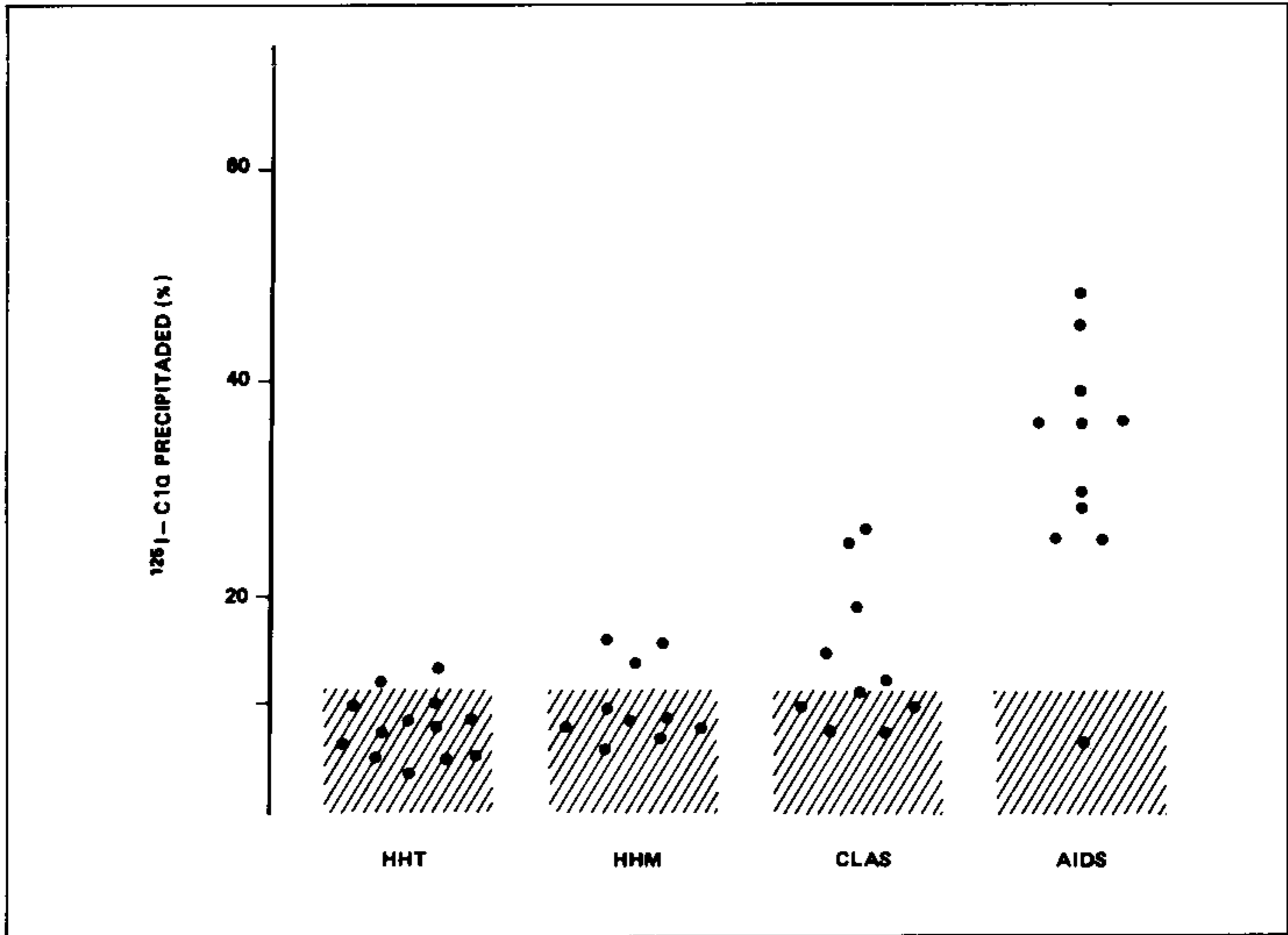


Fig. 3: circulating immune complex levels in patients with AIDS and CLAS by the I^{125} -Clq-binding assay. The normal range (90th percentile) in the HHT group is indicated by the hatched areas. HHT, healthy heterosexuals; HHM, healthy homosexual males; CLAS, chronic lymphadenopathy syndrome; AIDS, acquired immunodeficiency syndrome.

Suggestive findings of polyclonal B-cell activation (PBA), such as increased plasma immunoglobulins and high levels of CIC (Lane et al., 1983; Pahwa et al., 1984; Euler et al., 1985), were also observed. The low response to PWM is also compatible with a PBA, since it has been described that B-cells, when in a preactivated state *in vivo*, are refractory to *in vitro* stimulation by B-cell mitogens such as PWM (Lane et al., 1983). The mechanisms that induce PBA are still not completely understood. It is suggested that chronic antigenic stimulation by HIV may be a contributing factor for the development of PBA in AIDS (Yarchoan et al., 1986). In addition, PBA could also result from antigens derived from the opportunistic infections (Lane et al., 1985; Yarchoan et al., 1986), often seen in these patients.

Two out of 15 (13.3%) AIDS patients were seronegative in IIF, ELISA, DOT-ELISA and WB, and had elevated amounts of plasma CIC. This absence of detectable antibody may be

due to the presence of high numbers of virus replicating in the peripheral lymphocytes leading to antigen-excess conditions, which would account for the presence of HIV antigen-containing CIC. Alternatively, since these patients were at a final stage of the disease, and did not have increased titers of IgG, the negative serology could possibly reflect insufficient levels of specific circulating IgG antibodies due to B-cell exhaustion.

It has been suggested that immunologic alterations in homosexual men render these individuals more susceptible to the HIV. These immunologic abnormalities could result from others cofactor(s) such as infections with cytomegalovirus or hepatitis B and/or exposure to alloantigens in semen (Anderson & Tarter, 1982) or recreational drugs (Goedert et al., 1982). Recently, it was described the absence of cellular immunity abnormalities in HIV seronegative homosexual men (Dobozin et al., 1986). However, our data showed that some

HIV seronegative healthy homosexuals displayed a significant cellular immunity depression.

It seems that the epidemiologic distribution of AIDS in South and Southeast Brazil is similar to that in the developed countries (Curran et al., 1985; Ministério da Saúde, 1987; Galvão-Castro et al., 1987). Indeed, 86.6% of our cases were in homosexual and bisexual men. These results, however, differ from those obtained in Africa, where there is an almost equal disease distribution in male and female populations (Piot et al., 1984; Quinn et al., 1987).

The prevalence of AIDS in other areas of Brazil, is still low, nevertheless, some of these areas have several characteristics of some countries in Africa. Part of the population is of African origin; the climate is tropical; there is a high incidence of parasitic diseases and the socioeconomic level is low. It seems that potential activators *in vivo* such as cytomegalovirus, hepatitis B virus, and herpes simplex virus could induce HIV replication (Ho et al., 1987). Thus, we should taken into account the possible role of endemic parasitic diseases as activators of viral replication in these areas. It is possible that the pattern of spread of HIV infection in these areas will follow the pattern seen in Africa.

RESUMO

Alterações imunológicas na Síndrome de Imunodeficiência Adquirida em pacientes do Rio de Janeiro, Brasil. O perfil imunológico de 15 pacientes com Síndrome de Imunodeficiência Adquirida (AIDS) e 11 com Síndrome de Linfadenopatia Crônica, foram estudados. Os pacientes com AIDS mostraram reduzida percentagem de linfócitos T (CD3) totais e T auxiliares (CD4), aumento relativo no número de linfócitos T-supressores (CD8) e uma marcante inversão na relação T-auxiliares/supressores (CD4/CD8). A resposta linfoproliferativa para PHA, ConA, PPD e PWN, estava diminuída. Foi também observado hipergamaglobulinemia e níveis aumentados de complexos imunes circulantes. Os pacientes com Síndrome de Linfadenopatia Crônica também mostraram importantes alterações imunológicas, mas não tão intensas como nos de AIDS. Estes dados são similares aos observados nos Estados Uni-

dos e na Europa.

Palavras-chave: AIDS – alterações da resposta imune humoral e celular.

ACKNOWLEDGEMENTS

We wish to tanks, Dr Euclides Aires-de-Castilho for this assistance in the statistical analysis and Dr Lain Carlos Pontes-de-Carvalho for reviewing this manuscript.

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