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QUANTIFICATION OF LYMPHOCYTE SUBSETS IN AIDS ASSOCIATED WITH SUSPECTED CYTOMEGALOVIRUS INFECTION*

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ABSTRACT: The present study investigates the number of immunoregulatory cells in 55 patients suspected of Acquired Immunodeficiency Syndrome (AIDS) associated with cytomegalovirus (CMV) infection. Patients were divided into three groups: RISK (13 cases), LAS/ARC (21 cases) and AIDS (21 cases) according to epidemiological and clinical findings and using CDC criteria. The CMV infection was suggested based on clinical and serological evaluations. No significant difference in CMV seropositivity was observed among the groups (66.7% to 77.0%), although a diminished number of cases 3 (14.3%) with CMV-IgM antibody was detected in AIDS group. Phenotypic analysis of leukocytes, lymphocytes and lymphocyte subsets was realized. The results obtained were similar between CMV-seropositive and CMV-seronegative patients in each group studied. On the other hand, the number of T lymphocytes and T helper/inducer lymphocytes were markedly diminished in the LAS/ARC and AIDS groups. The number of T suppressor/ cytotoxic lymphocytes was increased in all groups analysed. These alterations in T lymphocyte subsets are responsible for the reversal ratio observed in these patients.

DESCRIPTORS: Acquired Immunodeficiency Syndrome (AIDS); cytomegalovirus (CMV); human immunodeficiency virus (HIV); lymphadenopathy syndrome/AIDS-related complex (LAS/ARC); lymphocyte subsets; CMV-IgG-antibody; CMV-IgM-antibody.

INTRODUCTION

Infection by the HIV virus results in damaged of specific cells, notably the lymphocytes population wich display surface CD4 marker as well as certain cells within the central nervous system[21]. Moreover, HIV can productively infect macrophages and monocytes, monocyte cell lines, CD8 + cell line and certain B-cell lines, but little or no cytopathic effect is noted in these cells types, probably because of the low concentration of T4 surface protein.

The cytopathic effect of the HIV are observed after membrane fusion in virus entry and syncytia formation. These events occur via interation of T4 epitope cell membrane and the HIV envelope glycoprotein (gp 120), that can be expressed on virus and infected cells[27].

These reactions lead to cell death by alteration of cell surface functions and loss of membrane integrity and swelling until disruption[13,22]. Because of the tropism of the virus to the central immunoregulatory cell (T helper/inducer lymphocyte), all immunological functions are altered which predispose the appearence of opportunistic infections observed in AIDS[15,19]. In immunosuppressed patients the cytomegalovirus (CMV) infections are frequent. The usual clinical and laboratorial manifestations observed in normal individuals are an infectious mononucleosis — like syndrome

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with the presence of atypical lymphocytosis in the peripheral blood and the absence of heterophils antibodies in the sera. The course of the disease is normally beningn although the CMV remain latent during the life of the host. In cases of immunosuppression, especially in account of damage of cell-mediated immunity, the virus may reactivate. In AIDS patients the virus reactivation is related to a variety of clinical syndromes including chorioretinitis, esophagitis, colitis, pneumonia, encephalitis and adrenalitis[7].

The present work has the aim of investigates the number of immunoregulatory cells in AIDS patients associated with CMV serological status.

PATIENTS AND METHODS

The study population consisted of 55 men aged 22 to 57 years (median 32) attended at Programme for AIDS Control and Prevention — Secretary of Health of São Paulo State with suspicion of AIDS associated with clinical CMV infection.

Patients were grouped as RISK, LAS/ARC and AIDS according to epidemiological and clinical features, using the definitions established by the Atlanta Center for Disease Contro[5,6]. The RISK group (13 asymptomatic male individuals) consisted of 3 heterosexual, 6 homosexual and 4 bisexual AIDS contacts, while the LAS/ARC group (21 cases) was composed of 19 homosexual and 2 bisexual men with persistent unexplained lymphadenopathy and others non specific clinical manifestations (fever, malaise, diarrhoea, weight loss). The AIDS group (21 cases) comprised 3 heterosexual, 13 homosexual and 5 bisexual men with opportunistic infections and/or Kaposi's sarcoma.

The clinical manifestations of CMV infection observed in these patients included chorioretinitis, esophagitis, pneumonia and infectious monocucleosis-like syndrome without heterophil antibodies.

Blood samples were collected and refered to the Instituto Adolfo Lutz for immunological evaluation. Inactivated sera were tested for IgG and IgM specific antibodies to CMV by an indirect fluorescent antibody test (IgM-IFA to CMV, VIRGO Reagents, Electro-Nucleonics Inc., Maryland, USA), and by complement fixation test according to methodology previously described[20] using as antigen Herpes group reagent (BEHRING, Merburg, West Germany). In these assays, titters equal or higher than 8 were considered positive.

The number of leukocytes, monocytes and lymphocytes were determined in heparinized blood using hemocytometer counter and Leishman stain smear. Monocuclear cells were separated on Ficoll-Hypaque gradient[3] and the number of T and B lymphocytes was obtained by means of the resette method using sheep red blood cells and zymosan-complement complex[1].

Direct immunofluorescence technique previously described[1]. was employed to determine the percentage of lymphocyte subsets using monoclonal antibodies conjugated with fluorescein isothiocyanate from Becton-Dickinson Lab. USA. (Leu-3a for CD4 cell surface antigen — Helper/inducer T lymphocyte and Leu-2a for CD8 cell surface antigen — suppressor/cytotoxic T lymphocyte). The samples were analysed for immunofluorescence using a Zeiss epifluorescent microscope (Carl Zeiss Inc., West Germany) at 1000 x.

Statistical analysis of the data were performed according to Mann-Whitney, Kruskal-Wallis tests and complemented by Dunn multiple comparison test[14,25].

RESULTS

Of the 55 male patients in this study, 38 were homosexual, 11 bisexual and only 6 were heterosexuals. The patients' age ranged from 22 to 57 years with a medium of 32 years.

After collecting information about social and clinical data, the population was divided in three groups: RISK, LAS/ARC and AIDS.

TABLE 1

Number of CMV—positive sera detected by complement fixation (CF) and immunofluorescence (IF) tests from RISK, LAS/ARC and AIDS groups.

Group (n)	CF		IF		CF and IF		Total	
	n	(%)	n	(%)	n	(%)	n	(%)
RISK (13)	3	(23.0)	3	(23.0)	4	(31.0)	10	(77.0)
LAS/ARC (21)	3	(14.3)	4	(19.1)	7	(33.3)	14	(66.7)
AIDS (21)	6	(28.6)	3	(14.3)	5	(23.8)	14	(66.7)

TABLE 2

Characterization of cells phenotypes from peripheral blood leukocytes in RISK, LAS/ARC, and AIDS groups according to serological CMV results.
Results are reported as medium values of the counts and the compared using Mann-Whitney analysis.

	RISK		LAS/ARC		AIDS	
	neg	pos	neg	pos	neg	pos
Leukocytes/mm^3	6900	6920	4920	5290	4460	4950
(n)	(3)	(10)	(7)	(14)	(7)	(14)
Lymphocytes/mm3	2240	2530	1810	2080	1505	1813
(n)	(3)	(10)	(7)	(14)	(7)	(14)
Monocytes/mm^3	229	332	214	237	172	189
(n)	(3)	(10)	(7)	(14)	(7)	(14)
%T Lymphocytes	55	63	57	63	62	55
(n)	(3)	(10)	(7)	(14)	(6)	(14)
T Lymphocytes/mm^3	2271	1596	1034	1339	926	1025
(n)	(3)	(10)	(7)	(14)	(6)	(14)
%B Lymphocytes	14.5	17.0	16.0	14.0	18.0	16.0
(n)	(2)	(10)	(7)	(13)	(7)	(12)
B Lymphocytes/mm^3	352	396	281	288	234	303
(n)	(2)	(10)	(7)	(13)	(7)	(12)
CD4 + Cells	33	26	18	21	21	15
(n)	(3)	(7)	(3)	(10)	(5)	(9)
CD8 + Cells	25	36	42	41	38	40
(n)	(3)	(7)	(3)	(10)	(5)	(8)
CD4/CD8 ratio	1.43	0.89	0.43	0.54	0.72	0.58
(n)	(3)	(7)	(3)	(10)	(5)	(9)

TABLE 3

Comparison of the cells phenotypes results obtained from the three groups studied
Results expressed as median values of the counts. P 0.05 as compared among the groups by Kruskal-Wallis analysis of variance and complemented by Dunn multiple comparisons test.*

	RISK X LAS/ARC		RISK X AIDS		LAS/ARC X AIDS	
Leukocytes/mm^3	6910	5170*	6910	4790*	5170	4790
Lymphocytes/mm^3	2470	1990	2470	1660	1990	1660
Monocytes/mm^3	308	229	308	183	229	183
%T lymphocytes	61	61	61	57	61	57
T Lymphocytes/mm^3	1752	1232	1752	996*	1237	996
%B Lymphocytes	16	15	15	17	15	17
B Lymphocytes/mm^3	389	286	389	278	286	278
CD4 + Cells	28	20	28	17*	20	17
CD8 + Cells	33	42	33	40	42	40
CD4/CD8 ratio	1.05	0.52	1.05	0.63*	0.52	0.63

CMV antibodies were present in 38 (69.1%) from 55 patients, and there is no significant difference when analysed statistically each group, separately (table 1).

Similar statistical results were observed when leukocytes, lymphocytes and monocytes were determined in CMV-seropositive and CMV-seronegative patients (table 2).

On the other hand, when compared cells phenotype in peripheral blood of AIDS and LAS/ARC groups with the RISK one, it was possible to demonstrate difference among them (table 3).

DISCUSSION

Several reports described a severe impairment of cellular immunity response in AIDS associated with an imbalance between helper and suppressor T lymphocytes[2,11,15,16,17,18,19].

Others disease states have been associated with alteration in immunoregulatory system with reversal ratio of CD4/CD8 lymphocytes as shown in CMV-mononucleosis, Epstein-Barr virus mononucleosis, hepatitis B and autoimmune diseases[4,9,23,24]. In such viruses during the acute phase of the disease occurs and elevation in the number of T suppressor/cytotoxic lymphocytes[4,11,23].

The CMV infection seen in AIDS patients is usually a reactivation of a childhood infection that was controlled, until HIV seriously destroyed the patients' immune system.

The ongoing presence of HIV overstimulates B lymphocytes that remains in a state of chronic activation[22]. The antibodies produced include some antibodies that combat current infections.

The activation of a great number of B lymphocytes diminishes the population of resting cells that are capable of produce antibodies in response to new pathogens. Consequently, the primary immune response is compromised leading to a low production of specific IgM antibodies[22].

In this study it was possible to demonstrate antibodies to CMV in 66.7% to 77.0% of the patients, and no difference was observed among the RISK, LAS/ARC and AIDS groups. These results contrast with the data previously reported, where in male homosexual and patients with AIDS, the prevalence of CMV infections was around 90%[7,9,17].

When IgM antibody was analysed separately, a low number or seropositivity in AIDS patients was observed. Similar results have been confirmed by others investigators[8,10].

On the other hand, one reason of the reduced IgM antibodies detected in these patients was due a large quantities of inespecific antibodies presenting in the patients' sera, which mask the IgM determination. In order to avoid this pitfall, a sera adsortion using anti-IgG antibody affinity column could be used to improve the detection of IgM-CMV antibody[26].

Although the detection of CMV antibodies has not been employed for diagnosis of a current infection, it provides a laboratory support to confirm a clinical suspicion of the disease.

The best criterion used to diagnose CMV infection requires the detection of the virus from the suspicious materials, employing culture methods or antigen detection techniques[7,8,12,26]. The positivity of these methods not prove the specific clinical syndrome, since virus and antigenic particles may be eliminated from urine, blood and genital fluids during a long period after primary and recurrent infections.

Serological conversion is the diagnostic means in nonimmunocompromised patients, but in cases of AIDS this is not true, considering that they are seropositive for CMV before contract HIV infection[8].

Despite the difficult, in this work was considered active infection due CMV, when taken together clinical manifestations and serological positive results.

These data showed the number of immunoregulatory cells in AIDS patients seropositive and seronegative for CMV. It was impossible establish a characteristic lymphocyte subsets imbalance for these associated diseases. The cellular status of these cases was the same that was observed on the others AIDS' cases associated with certains opportunistic infections[2,19].

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CATERINO-de-ARAUJO, A.; SANTOS-FORTUNA, E. de los & UEDA, M. — Determinação de subpopulações linfocitárias na AIDS associada com provável infecção pelo Citomegalovírus. *Rev. Inst. Adolfo Lutz*, 50 (1/2): 285-290, 1990.

RESUMO: Este trabalho teve como objetivo determinar o número de células imuno-reguladoras em 55 casos suspeitos de Síndrome de Imunodeficiência Adquirida (AIDS) associada com infecção citomegálica. Usando os critérios estabelecidos pelo CDC, os pacientes foram divididos em três grupos de acordo com dados epidemiológicos e clínicos: RISCO (13 casos), LAS/ARC (síndrome da linfoadenopatia / complexo relacionado à AIDS) (21 casos) e AIDS (21 casos). A infecção pelo Citomegalovírus foi sugerida com base nas avaliações clínicas e laboratoriais. Nenhuma diferença significativa foi observada na soropositividade para o CMV entre os grupos analisados (66,7% a 77,0%) , apesar do grupo AIDS apresentar um pequeno número de casos 3 (14,3%) com anticorpo positivo para o CMV de classe IgM. A análise fenotípica dos linfócitos desses pacientes mostrou resultados semelhantes entre os grupos com sorologia positiva e negativa para o CMV. Por outro lado, o número de linfócitos T auxiliadores/indutores mostraram-se marcadamente diminuídos nos grupos LAS/ARC e AIDS. O número de linfócitos T supressores/citotóxicos encontravam-se aumentados em todos os grupos analisados. Essas alterações nas subpopulações linfocitárias foram responsáveis pela inversão da relação linfócitos T auxiliadores/linfócitos T supressores observada nesses pacientes .

DESCRITORES: Síndrome de Imunodeficiência Adquirida (AIDS), Citomegalovírus (CMV), Vírus de Imunodeficiência Humana (HIV), síndrome da linfoadenopatia / complexo relacionado à AIDS (LAS/ARC), subpopulações de linfócitos, anticorpo para CMV de classe IgG, anticorpo para o CMV de classe IgM.

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