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Immunologic abnormalities related to antigenaemia during HIV-1 Infection

P. DE PAOLI, S. BATTISTIN, MARINA CROVATTO, MARIA LUISA MODOLO, A. CARBONE,*
U. TIRELLI† & G. SANTINI *Department of Microbiology and Immunology, General Hospital, Pordenone,*
**Division of Pathology & †Division of Medical Oncology, Centro di Riferimento Oncologico, Aviano, Italy*

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SUMMARY

The expression of phenotypic markers on T and B lymphocytes in long-term human immunodeficiency virus type 1 (HIV-1) seropositive, antigen negative patients, in seropositive, antigen positive individuals without AIDS and in seronegative intravenous drug abusers was examined by two colour flow cytometry. Seropositive, antigen postive patients showed decreased CD4$^+$ lymphocyte numbers, causing lower CD4/CD8 ratios when compared to seropositive, antigen negative subjects. While CD4 CDw29$^+$ (4B4) lymphocytes are selectively reduced in seropositive, antigen negative individuals, both CD4 CDw29$^+$ and CD4 CD45R$^+$(2H4) lymphocytes are decreased when antigenaemia is present. An increased percentage of CD3 HLA DR$^+$ activated T lymphocytes and of CD20$^+$(B1) Leu 8 negative activated B cells was seen in HIV-1 seropositive antigen positive patients. These results demonstrate that, in long-term seropositive individuals, antigenaemia is associated with peculiar phenotypic changes of lymphocyte subsets.

Keywords HIV-1 antigen CD4 CDw29 CD45R lymphocytes

INTRODUCTION

Human immunodeficiency virus type 1 (HIV-1) causes the acquired immunodeficiency syndrome (AIDS) (Wong Staal & Gallo 1985; Lane *et al.*, 1985a; Melbye *et al.*, 1986). This virus has a tropism for CD4$^+$ lymphocytes and monocytes. After infection the virus integrates a copy of its genetic material into the host cell genoma and, when lymphocytes are activated by unrelated antigens, viral particles are produced, resulting in cell lysis (Klatzmann & Gluckmann, 1986; Quinnan *et al.*, 1985).

HIV-1 infection is characterized by profound abnormalities in cellular immune function. CD4$^+$ lymphocytes are reduced, CD8$^+$ cells retain normal or increased values and a progressive inability to respond to soluble antigens or to allostimulation has been described (Lane *et al.*, 1985b; Lewis *et al.*, 1985; Shearer *et al.*, 1986; Quinn *et al.*, 1987).

In-vivo activation of B lymphocytes results in the increased production of polyclonal immunoglobulins (Lane *et al.*, 1983; Sjamsoeddin Viser *et al.*, 1987).

HIV-1 antigen has been detected in the sera of patients who have recently seroconverted, in AIDS patients and sometimes in long-term seropositive individuals without AIDS (Goudsmith *et al.*, 1986; Kenny *et al.*, 1987). HIV-1 antigenaemia seems to be related to a rather bad prognosis (Kenny *et al.*, 1987) but the pathogenetic mechanisms have not been investigated.

We have examined the phenotypic profile of lymphocyte subsets in intravenous (i.v.) drug abusers.

Our results indicate that antigenaemia is accompanied by low CD4$^+$ cell numbers, reduced CD4/CD8 ratios and increased numbers of activated lymphocytes.

Correspondence: Dr. P. De Paoli, MD, Department of Microbiology and Immunology, General Hospital, I 33170 Pordenone, Italy.

MATERIALS AND METHODS

Patients and study individuals

Intravenous (i.v.) drug abusers without AIDS were included in our study, which was initiated in April 1984. The patients designated long-term seropositive had antibodies to HIV-1, as measured by ELISA and Western blot, at first examination during 1984 or 1985 and thus had been infected for at least two years. Thirty subjects (13 had lymphadenopathy, the others were asymptomatic) did not have antigenaemia. In the sera of nine patients (six had lymphadenopathy, the others were asymptomatic) we could detect HIV-1 antigenaemia. Patients who developed antibodies to HIV-1 or AIDS while under observation were not included. Controls included asymptomatic seronegative i.v. drug abusers ($n=45$) and healthy subjects ($n=20$) who belonged to the same age group (range 20–40 years).

Lymphocyte subpopulations

Peripheral blood anticoagulated with heparin was sedimented in Plasmagel for 30 min at 37°C. The leucocyte rich buffy coat was used to measure lymphocyte subsets. Briefly, 100μl of the cell suspension was separately incubated with FITC labelled CD3 (OKT3, Ortho, Milan), CD4 (OKT4, Ortho, Milan), CD8 (OKT8, Ortho, Milan) Leu 7 (Becton Dickinson, Milan) and CD20 (B1, Coulter, Milan) monoclonal antibodies. After incubation and further washings with PBS, phycoerythrin labelled CDw29 (4B4, Coulter, Milan), CD45R (2H4, Coulter, Milan), CD11b (Leu 15, Becton Dickinson, Milan), CD8 (Leu 2a, Becton Dickinson, Milan) and HLA DR (Becton Dickinson) were added to obtain the appropriate combinations (see Results). Isotype controls labelled with FITC or PE were used to assess non-specific binding. Lymphocytes were washed in PBS and immunofluorescence was measured in a FACS 440 cytofluorograph equipped with a 5 W argon laser and a Consort 40 computer. Double fluorescence was calculated by computer assisted analysis after electronic subtraction of the signals to obtain true fluorescein and phycoerythrin outputs in the respective detectors. Lymphocyte counts were obtained by standard haemocytometric techniques.

HIV-1 serology

The presence of antibodies to HIV-1 was determined by using an enzyme linked imunoassay (HTLV III EIA, Abbott, Rome) and by Western blot (Tsang, Peralta & Simons, 1983; Popovic *et al.*, 1984), using antigen blotted nitrocellulose strips (Dupont, Rome). Briefly, 20 μl of diluted samples was incubated overnight at room temperature with these strips. Visualization of human antibodies specifically bound to HIV-1 proteins was accomplished by serial reactions with biotin-conjugated goat anti-human IgG antiserum, horseradish peroxidase (HRP) conjugated avidin and the HRP substrate 4-chloro-1-naphthol. A specimen was considered positive when a band at p24, p31 and either gp41 or gp 160 were present. None of the patients was p24 negative.

HIVI-1 antigen determination

Samples were assayed in a solid phase immunoassay (HTLV III antigen EIA, Abbott, Rome) in which polyclonal antibodies to HIV-1 were used as capture. Briefly, 200 μl of each specimen was incubated at room temperature with polystyrene beads coated with human anti-HIV-1 IgG. After washings the beads were incubated with rabbit anti-HIV-1 antiserum for 4 h at 40°C. After further washings, an HRP labelled goat anti-rabbit antiserum was added (2 h at 40°C). Colour was developed with *o*-phenylenediamine as substrate and absorbance was measured at 492 nm. A specimen was considered positive when the optical density was 0·050 plus the mean of five negative controls (Allain *et al.*, 1987). All positive sera were examined by a neutralization test (HTLV III antigen neutralization test, Abbott, Rome). When the absorbance reduction of the neutralized specimen was 50% or more, as compared to a matched non-neutralized control, the specimen was considered positive.

Statistical analysis

The results are presented as mean ± standard deviation. Significance was tested using Student's *t*-Test.

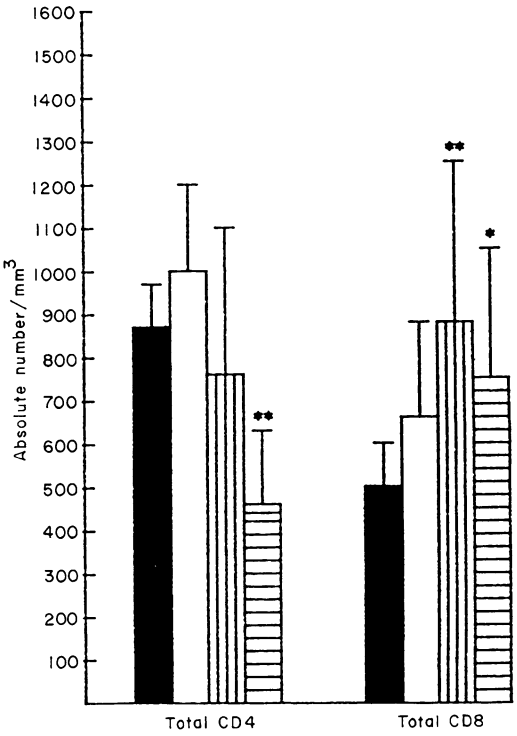

Fig. 1. Absolute value of CD4$^+$ and CD8$^+$ cells in peripheral blood of the indicated groups: mean ± 1 s.d.; * P 0·05, ** P 0·01, ■ healthy controls; □ seronegative, ▥ seropositive, Ag negative, ▤ seropositive, Ag positive.

RESULTS

CD4 and CD8 levels in HIV-1 infected i.v. drug abusers

One-colour immunofluorescence staining of lymphocytes from i.v. drug abusers and healthy controls was performed to enumerate CD4 and CD8 levels. The long-term seropositive patients were stratified into two groups: those who were seropositive, but antigen negative ($n=30$) and those who were antigen positive ($n=9$).

Figure 1 shows the mean values for CD4$^+$ and CD8$^+$ subsets in the five groups. Seronegative individuals did not differ from healthy controls. The seropositive, antigen negative patients had increased numbers of CD8$^+$ cells. When seropositive, antigen positive i.v. drug abusers were considered, both CD4$^+$ and CD8$^+$ lymphocytes showed statistically significant differences compared to controls.

Alterations in functional subsets of CD4$^+$ and CD8$^+$ lymphocytes

The monoclonal antibodies to CDw29(4B4) and to CD45R(2H4) (Morimoto *et al.*, 1985a; b) were used to determine whether HIV-1 infection results in selective alterations of distinct CD4$^+$ subsets. Table 1 shows the mean values of percentages and absolute numbers of lymphocyte subsets in the four groups studied. The CD4 CDw29$^+$ population was depleted in seropositive, antigen negative patients, but when antigenaemia is present the CD4 CD 45R$^+$ subset was reduced also.

The expression of CD11b and Leu 7 surface antigens was used to delineate CD8$^+$ subsets (Clement, Grossi & Gartland, 1984. Lewis *et al.*, 1985). CD8 Leu 7$^+$ cells were increased in

Table 1. Percentages and absolute number (in parentheses) of $CD4^+$ and $CD8^+$ subpopulations

	$CD4^+$ $CDw29^+$	$CD4^+$ $CD45R^+$	$CD8^+$ Leu 7^+	$CD8^+$ $CD11b^+$	CD4/CD8
Healthy control	23·5±6·0 (485±120)	15·3±7·0 (320±165)	4·7±2·0 (96±41)	3·0±3·0 (62±25)	1·73±0·55
Seronegative risk group members	18·4±4·7 (460±132)	17·2±9·0 (445±230)	4·6±2·6 (115±25)	6·6±4·0 (164±112)	1·56±0·35
Seropositive, antigen negatives	12·0±4·0† (295±180)†	15·9±6·0 (375±200)	10·7±7·0* (196±130)*	9·2±8·0* (170±145)*	0·91±0·4*
Seropositive, antigen positives	10·9±3·0† (184±60)†	11·8±5·0* (200±90)*	13·3±8·0† (284±200)*	4·9±3·0 (86±63)	0·65±0·2†

* $P<0.05$, † $P<0.01$

Table 2. Percentages and absolute number (in parentheses) of total and activated ($CD3^+$HLA DR^+) T cells and of total and activated ($CD20^+$ Leu 8 negative) B cells.

	$CD3^+$	$CD3^+$HLA DR^+	$CD20^+$	$CD20^+$Leu 8^-
Seronegatives	73·1±8·0 (1710±210)	2·2±0·5 (57±15)	8·2±1·2 (200±60)	1·0±0·4 (25±10)
Seropositives, antigen negatives	77·6±8·0 (1670±550)	3·8±2·0 (75±40)	6·9±3·0 (157±60)	2·0±1·0 (48±24)
Seropositives, antigen positives	74·5±9·0 (1270±410)*	7·2±3·0† (120±40)†	8·0±2·0 (123±40)*	4·7±3·0† (70±40)†
Healthy controls	(73·1±8·0 (1650±310)	2·0±1·0 (40±15)	7·7±1·9 (158±39)	0·9±0·5 (18±10)

* $P<0.05$, † $P<0.01$

seropositive individuals, particularly when antigenaemia was present. CD4/CD8 ratios were more reduced in seropositive, antigen positive compared to the antigen negative subjects.

Increased numbers of activated lymphocytes

Two-colour flow cytometry, utilizing CD3 and anti-HLA DR monoclonal antibodies, was used to assess T cell activation. Absolute numbers of activated T cells were increased in seropositive, antigen positive individuals, when compared to the other three groups (Table 2). The absolute number of activated B cells, studied by CD20 and Leu 8 monoclonal antibody combinations was also evaluated. A significant increase in the fraction of B cells lacking Leu 8, a cell surface marker associated with resting B cells (Kansas *et al.*, 1985), was seen in seropositive individuals, the phenomenon being more pronounced in antigen positive subjects (Table 2).

DISCUSSION

This study provides new information about the immune abnormalities in HIV-1 infected intravenous drug abusers. In long-term seropositive individuals without AIDS antigenaemia is correlated with peculiar immunologic abnormalities. As outlined in Fig. 1 and Table 1, HIV-1 seropositive, antigen positive subjects had reduced $CD4^+$ cells, slightly increased $CD8^+$ lymphocytes and their CD4/CD8 ratio decreased to 0·65±0·2. Seropositive, antigen negative individuals had a ratio of 0·91±0·4 mainly because of raised $CD8^+$ cell numbers. Recent data suggest an association between antigenaemia and depletion of $CD4^+$ cells and a relationship to a poorer prognosis (Pedersen *et al.*, 1987).

Seropositive, antigen negative patients show how a decrease of the CD4 $CDw29^+$ population and this observation agrees with our previous finding that this subset is reduced in the lymph-nodes of HIV-1-infected drug abusers withthe prolonged generalized lymphadenopathy syndrome (De Paoli *et al.*, 1987). In homosexual men low CD4 numbers could not be attributed to a defined $CD4^+$ subpopulation (Gupta, 1987; Giorgi *et al.*, 1987). These studies refer to different risk groups and no data about the presence of HIV-1 antigens were reported. When antigenaemia is present the reduction of $CD4^+$ lymphocytes also affects the reciprocal $CD45R^+$ subset. A similar picture is observed during full blown AIDS (P. De Paoli, unpublished observations). The absence of antigen in the sera of seropositive individuals may reflect a latent phase of HIV-1 infection. During this phase CD4 $CDw29^+$ cells are deeply reduced. It has been suggested that transition from a latent to an active state of infection is characterized by augmented expression of HIV-1 genes, that is the presence of HIV-1 antigen (Lange *et al.*, 1986). During this phase both CD4 $CDw29^+$ and CD4 $CD45R^+$ cells were reduced in the peripheral blood of our patients. The reasons for this behaviour are unknown, but it is possible that different surface molecules on $CD4^+$ lymphocytes modulate cell protection/susceptiblity to HIV-1 infection or cell damage. Serra *et al.* (1988) proposed that CD45R cells are an immature stage in a lineage that culminates in $CDw29^+$ expression. The degree of cell maturity could be also relevant with respect to retroviral infection. Increased CD8 cell numbers could suggest a

response to HIV-1 infected cells. In seropositive individuals the proportions and numbers of CD8 Leu 7+ lymphocytes are increased, especially when antigenaemia is present (Table 1). However this finding is not specific for HIV-1 infection and may be a general response to viruses or other chronic antigenic stimulations (Leroy *et al.*, 1986; Gratama *et al.*, 1987). Elevated numbers of activated T and B cells were present in the blood of seropositive, antigen positive subjects (Martinez Maza *et al.*, 1987).

The factors responsible for HIV-1 infection and subsequent development of disease are multiple and complex but it has been demonstrated that activated lymphocytes are more susceptible to productive infection with HIV-1 than are resting cells (Montagnier *et al.*, 1985; McDougal *et al.*, 1986).

Taken collectively our data suggest that long-term HIV-1 seropositive i.v. drug abusers with antigenaemia had peculiar immunologic features characterized by reduced CD4 cell numbers, very low CD4/CD8 ratios and consistent lymphocyte activation. Studies are underway to establish if antigenaemia and the described lymphocyte subset alterations constitute bad prognostic factors.

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