Clin Exp Immunol 1994; 95:436–441

Selective increase of activation antigens HLA-DR and CD38 on $CD4^+CD45RO^+$ T lymphocytes during HIV-1 infection

L. KESTENS, G. VANHAM, C. VEREECKEN, M. VANDENBRUAENE*†, G. VERCAUTEREN*, R. L. COLEBUNDERS*† & P. L. GIGASE *Laboratory of Pathology & Immunology, *Microbiology and †Outpatient Clinic, Institute of Tropical Medicine, Antwerpen, Belgium*

(Accepted for publication 5 November 1993)

SUMMARY

Infection with HIV results in a progressive depletion of $CD4^+$ T cells and leads to significant *in vivo* lymphocyte phenotype changes. In this regard, the expression of HLA-DR and CD38 on $CD8^+$ T cells has been shown to increase dramatically with disease progression. We investigated the expression of both activation markers on $CD4^+$ T cells in HIV-1-infected subjects at different clinical stages of infection and compared the *in vivo* activation of $CD4^+$ T cells with parameters of viral activity and $CD8^+$ T cell activation. Fresh peripheral venous blood was obtained from 54 HIV-infected subjects and from 28 uninfected healthy controls. Three-colour immunophenotyping of the $CD4^+$ T cell subset showed that the proportion of $CD4^+$ T cells expressing HLA-DR (10% in HIV-negative controls) or CD38 (62% in HIV-negative controls) was higher in asymptomatic ($P < 0{\cdot}05$ for CD38) and symptomatic ($P < 0{\cdot}001$ for HLA-DR and CD38) HIV-infected subjects than in controls, whereas the proportion of $CD4^+$ T cells expressing CD45RO (54% in controls) remained relatively unchanged. Simultaneous expression of HLA-DR and CD38 on $CD4^+$ T cells increased from 2·3% in controls to 11% ($P < 0{\cdot}001$) in asymptomatic and 22% ($P < 0{\cdot}001$) in symptomatic HIV-infected subjects. This relative increase of CD38 and HLA-DR expression occurred mainly on $CD4^+$ T cells co-expressing CD45RO. Changes in expression of HLA-DR and CD38 on $CD4^+$ T cells correlated with similar changes on $CD8^+$ T lymphocytes, with the presence of HIV antigen in the circulation, and with the disease stage of HIV infection.

Keywords HIV-1 $CD4^+$ T cells HLA-DR CD38 CD45RO

INTRODUCTION

The progressive decline of the number of $CD4^+$ T lymphocytes in peripheral blood is regarded as one of the most reliable laboratory markers of disease progression in subjects infected with HIV-1 [1]. HIV-1 infection also induces T cell activation, and this is manifested by increased expression of several phenotypic markers, mainly within the CD8 lymphocyte subset. In particular, the expression of HLA-DR (MHC class II antigen) and CD38 increases dramatically on CD8 cells with disease progression [2–4]. In healthy HIV-negative individuals, CD38 is expressed on immature T cells and disappears after the transition from an unprimed ($CD45RA^+$) to a primed ($CD45RO^+$) cellular maturation stage [5]. However, CD38 is re-expressed on T cells upon *in vitro* activation [6]. After HIV infection, the absolute number of $CD4^+$ T cells decreases, but the ratio of memory or 'primed' ($CD45RO^+$) cells to naive or 'unprimed' ($CD45RA^+$) $CD4^+$ T cells appears to remain relatively unchanged [7–10]. So far, relatively few studies have addressed *in vivo* activation antigen expression on $CD4^+$ T cells of HIV-infected subjects. The present work was undertaken to assess the expression levels of HLA-DR and CD38 on $CD45RO^+$ and on $CD45RO^-$ $CD4^+$ T cells in peripheral blood from HIV-1-infected subjects using three-colour flow cytometry. The expression of these antigens on $CD4^+$ T cells was analysed at various HIV disease stages and was compared with the expression levels of the same antigens on CD8 cells. Serum levels of soluble CD8 (sCD8) were measured as a marker of activation of $CD8^+$ cells [11] and HIV antigen was measured in the serum as a marker of HIV activity.

Correspondence: Luc Kestens DrSc, Laboratory of Pathology & Immunology, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerpen 1, Belgium.

PATIENTS AND METHODS

Subjects

Fifty-four Caucasian patients with Western blot-confirmed HIV-1 infection and 28 HIV-negative healthy blood donor controls were enrolled (Table 1). In order to include patients from all clinical stages, comparable numbers of patients with a $CD4^+$ T percentage between 5% and 10%, between 10% and

Table 1. Study population, anti-retroviral treatment, HIV antigen and CD4 count

	HIV$^-$ (n=28)	WHO1 (n=27)	WHO2+3 (n=15)	WHO4 (n=12)
Male/female	NA	21/6	14/1	11/1
Median age (years)	NA	36	43	42
Received anti-retroviral treatment	—	6 (22%)	7 (47%)	12 (100%)
HIV antigen-positive	—	2 (7%)	9 (60%)	7 (58%)
Total lymphocyte count* (cells/mm^3)	1378 (1163–1723)	1957 (1644–2489)	1290 (906–1768)	1027 (893–1500)
CD4 percentage*	46 (40–50)	24 (18–33)	14 (7–32)	8 (5·6–13)
Total CD4 count* (cells/mm^3)	635 (476–774)	459 (307–637)	199 (6–400)	104 (45–208)

* Results are expressed as median and interquartile ranges (p25–p75).
NA, Not available.

20%, between 20% and 30% and with more than 30% were examined. HIV$^+$ subjects were clinically classified according to the criteria established by the World Health Organization [12]. Twenty-seven patients were asymptomatic or presented with lymphadenopathy (WHO stage 1), 15 were symptomatic non-AIDS patients (WHO stages 2+3), and 12 were AIDS patients (WHO stage 4).

Immunophenotyping of lymphocyte subsets

From each subject, fresh peripheral venous blood was obtained in 5-ml EDTA tubes and analysed within 4 h of sample collection.

In vivo CD4$^+$ T cell activation was examined by three-colour immunophenotyping using peridinyl chlorophyll-A protein (PerCP)-conjugated anti-CD4 (Leu-3) with various combinations of FITC-conjugated anti-HLA-DR and anti-CD45RO (UCHL1) and PE-conjugated anti-HLA-DR and anti-CD38 (Leu-17). CD8$^+$ cell activation was examined by two-colour lymphocyte immunophenotyping using anti-CD8 (Leu-2a-PE and Leu-2a-FITC) in combination with anti-HLA-DR-FITC and anti-CD38 (Leu-17-PE) respectively. All MoAbs were purchased from Becton Dickinson (Erembodegem, Belgium).

The lymphocyte staining was performed according to the whole blood lysing procedure and has been described elsewhere [3]. The stained samples were stored at 4°C and analysed using a FACScan flow cytometer with LYSYS software within 24 h after fixation. A live gate was set around the CD4$^+$ cluster in order to acquire a minimum of 1000 CD4$^+$ cells during cell acquisition. To prevent monocytes from being included in the analyses, only bright CD4$^+$ cells were considered.

Detection of total HIV-1 antigen

Plasma specimens were tested for the presence of HIV-1 antigen by ELISA (HIV Ag Innotest; Innogenetics, Antwerp, Belgium) according to the instructions of the manufacturer. HIV-1 antigen-negative samples were reassessed after acid dissociation of immune complexes [13,14]. Briefly, 100-μl plasma specimens were hydrolysed with 50 μl 1 N HCl and incubated for 1 h at 37°C. The samples were then neutralized with 50 μl 1 N NaOH and tested for the presence of HIV antigen.

Determination of soluble CD8 antigen

Soluble, cell-free CD8 antigen was quantified by ELISA (Cell-Free CD8 test kit; T-Cell Sciences, Inc., Cambridge, MA) according to the instructions of the manufacturer. Plasma stored at −20°C was thawed slowly and 5 μl were assayed undiluted. CD8 antigen reference standards provided in the kit were assayed in parallel to generate a standard curve of units of sCD8 per ml serum.

Statistical analysis

Differences between groups were tested for statistical significance using the non-parametric Wilcoxon two-sample test. Where appropriate, P values were corrected applying the Bonferroni adjustment for multiple comparisons. For each combination of activation antigens studied on CD4$^+$ T cells, the P value was multiplied by 4 to adjust for comparing results of the four dependent fluorescence quadrants. The 95% confidence intervals of the median were calculated using the formula: $1{\cdot}56(p75-p25)/SQRT(N)$ in which p75 and p25 represent the 75th and the 25th percentile about the median and N the number of subjects [15]. Correlation analysis was performed with the Spearman's rank-correlation test.

RESULTS

Subjects and treatment

The male to female ratio, the median age, the use of anti-retroviral treatment (zidovudine), the presence of HIV antigen in serum, the total lymphocyte count and the relative and absolute CD4$^+$ T cell counts are presented in Table 1.

Three-colour immunophenotyping of CD4 cells

HLA-DR expression was generally low in HIV-negative controls (median 10·4%), but 50% or more of the CD4$^+$ T cells expressed CD38 (median 62%) or CD45RO (median 54%) (Fig. 1).

All HIV$^+$ groups showed a relative increase of HLA-DR and CD38 expression on CD4$^+$ T cells compared with controls. HLA-DR and CD38 expression increased from WHO stage 1 to subsequent WHO disease stages. Conversely, the expression of CD45RO on CD4$^+$ T cells did not differ significantly between HIV-infected subjects and controls, and was only slightly increased in patients from the WHO stages 2, 3 and 4 (Fig. 1).

In HIV-negative control subjects, most CD4$^+$ T cells expressed CD38 but no HLA-DR (median 59%) or were negative for both markers (median 34%), whereas only a small proportion of CD4$^+$ T cells were CD38$^+$HLA-DR$^+$ (median 2·3%) or CD38$^-$HLA-DR$^+$ (median 3·8%) (Fig. 2a). The proportion of CD4$^+$ T cells that expressed CD38 alone in HIV$^+$

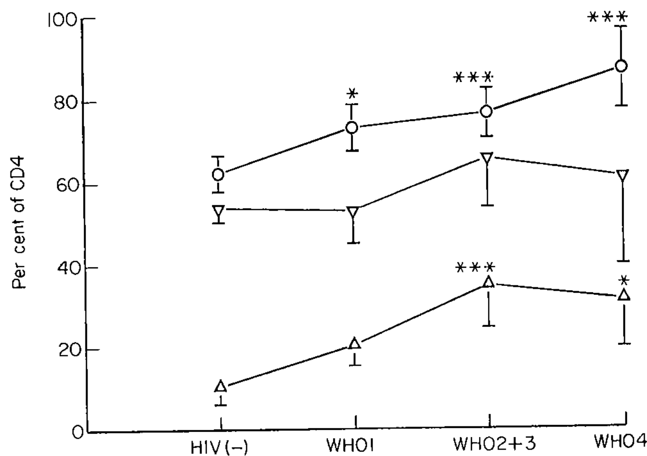

Fig. 1. Proportion of $CD4^+$ T cells that expresses HLA-DR (▵), CD45RO (▿) or CD38 (○) antigen in relation to the clinical stage of HIV infection. HIV(−), Uninfected controls; WHO1, asymptomatic HIV infection; WHO2+3, HIV-infected subjects with non-AIDS defining signs or symptoms; WHO4, AIDS patients. The results are presented as the median and the 95% confidence intervals of the median. Differences in expression levels were tested for statistical significance using the non-parametric Mann–Whitney *U*-test. $^*P<0.05$; $^{**}P<0.005$; $^{***}P<0.001$.

subjects remained unchanged throughout the various disease stages, but a significant proportional increase of $CD38^+HLA\text{-}DR^+$ on $CD4^+$ T cells was noticed. Although the $CD4^+$ T cells with the $CD38^-HLA\text{-}DR^+$ phenotype increased significantly ($P<0.001$), their relative quantitative importance remained low. Patients from WHO stages 2, 3 and 4 had a significantly larger proportion of $CD4^+$ T cells that were $CD38^+HLA\text{-}DR^+$ than subjects from WHO stage 1 ($P<0.01$).

The proportional increase of HLA-DR and CD38 expression on $CD4^+$ T cells was mainly observed on $CD4^+$ T cells expressing CD45RO (Fig. 2b and c, respectively), and for CD38 increased significantly from WHO stage 1 to WHO stages 2, 3 and 4 ($P<0.05$).

Dot plots of representative subjects from each group qualitatively illustrated that the increased expression of CD38 and HLA-DR on $CD4^+$ T cells at different clinical stages was almost exclusively restricted to the $CD4^+$ T cells expressing CD45RO (Fig. 3).

Correlation between markers of $CD4^+$ and $CD8^+$ T cell activation

A significant positive correlation was found between the increased expression of HLA-DR on $CD4^+$ and on $CD8^+$ T cells of HIV^+ subjects ($r=0.67$; $P<0.0001$). A similar correlation was observed for CD38 ($r=0.68$; $P<0.0001$).

The presence of soluble CD8 antigens in the plasma correlated significantly with the percentage and absolute number of activated $CD8^+HLA\text{-}DR^+$ T cells ($r=0.60$, $p<0.0001$; $r=0.55$, $P<0.0001$, respectively). The closest association between the expression of activation markers on $CD4^+$ T cells and the presence of soluble CD8 in the plasma was observed with the proportion of $CD4^+$ T cells expressing HLA-DR and CD38 simultaneously ($r=0.50$, $P<0.0001$).

Correlation between the presence of HIV antigen and activation of $CD4^+$ and $CD8^+$ T cells

HIV^+ subjects with detectable plasma levels of HIV antigen compared with those without soluble HIV antigen had significantly lower CD4 percentages (median 12% *versus* 23%, $P=0.001$) and absolute CD4 counts (median 199 *versus* 36[illegible], $P=0.0017$), significantly higher proportions of $CD8^+$ cells expressing CD38 (87% *versus* 64%, $P=0.0002$), significantly higher proportions of $CD4^+$ T cells that are $CD45RO^+HLA\text{-}DR^+$ (23% *versus* 16%, $P=0.027$), $CD45RO^+CD38^+$ (41% *versus* 23%, $P=0.001$) and $HLA\text{-}DR^+CD38^+$ (22% *versus* 12%, $P=0.0003$), but only slightly higher sCD8 levels (871 *versus* 739 units, $P=0.26$) and proportions of $CD8^+$ cells that express HLA-DR (65% *versus* 57%, $P=0.25$).

Anti-retroviral treatment and activation of $CD4^+$ and $CD8^+$ T cells

To study the effect of anti-retroviral treatment on HLA-DR and CD38 expression on $CD4^+$ and $CD8^+$ T cells, treated patients were matched with untreated individuals for their relative and absolute CD4 counts. Consequently, two equally sized groups of 13 individuals with a comparable degree of immunodeficiency could be assembled and compared. The median CD4 percentage was 20·3% in the treated group and 20·8% in the untreated group ($P=0.98$), whereas the corresponding absolute CD4 counts were 307 and 296 cells/μl respectively ($P=0.59$). In the treated and untreated group, CD4 cells expressed comparable levels of CD38 (75·9% *versus* 75·7%, $P=0.86$) and HLA-DR (22·3% *versus* 20·8%, $P=0.62$). Similar proportions of CD4 cells were $CD45RO^+HLA\text{-}DR^+$ (18% *versus* 19%, $P=0.47$), $CD45RO^+CD38^+$ (25·6% *versus* 28·7%, $P=0.82$) and $HLA\text{-}DR^+CD38^+$ (13·4% *versus* 13·6%, $P=0.70$). In both groups, comparable proportions of CD8 cells were $CD38^+$ (71·4% *versus* 68·4%, $P=0.70$) and $HLA\text{-}DR^+$ (54·1% *versus* 62·5%, $P=0.25$). In addition, the plasma levels of soluble CD8 were found to be similar (871 *versus* 874 units, $P=0.49$).

DISCUSSION

This study showed that the relative proportion of $CD4^+$ T cells expressing HLA-DR and CD38 is increased in HIV-seropositives and that the relative expression of both markers is higher in symptomatic and AIDS patients than in asymptomatic subjects. These changes were relatively small when expressed in proportion to the total lymphocytes, since they were largely compensated for by the progressive depletion of $CD4^+$ T cells. This is probably the reason why these changes were not recognized and reported earlier.

The proportion of $CD4^+$ T cells expressing HLA-DR and CD38 increased significantly with disease progression, and was almost exclusively confined to $CD45RO^+$ $CD4^+$ T cells, suggesting a proportional increase of activated 'primed' $CD4^+$ T cells or activated but not irreversibly switched to memory $CD4^+$ T cells [16]. The relative increase of $CD4^+$ T cells concomitantly expressing HLA-DR and CD38 seems to resemble previous observations within the CD8 subset [2–4]. The major difference between the two observations is that phenotypic changes within the CD8 subset represent mainly an expanding cell population, whereas the changes within the CD4 subset rather represent the phenotype of a subsiding subset of $CD4^+$ T cells.

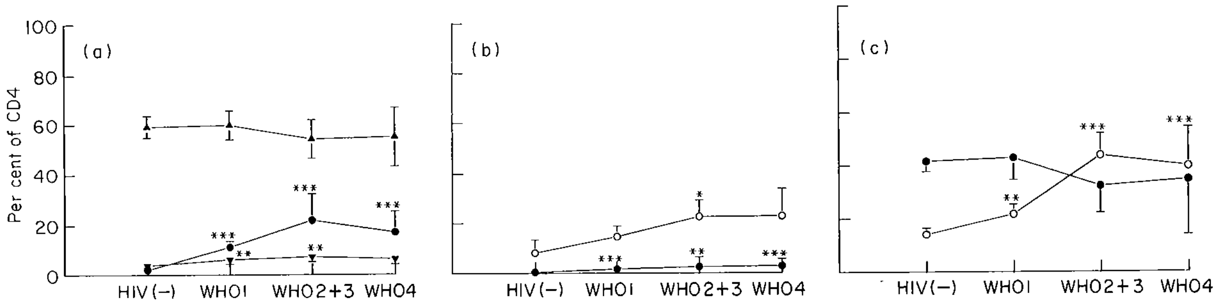

Fig. 2. Proportion of $CD4^+$ T cells that express (a) CD38 and HLA-DR, (b) HLA-DR and CD45RO, and (c) CD38 and CD45RO in relation to the clinical stage of HIV infection. HIV(–), Uninfected controls; WHO1, asymptomatic HIV infection; WHO2+3, HIV-infected subjects with non-AIDS defining signs or symptoms; WHO4, AIDS patients. The results are presented as the median and the 95% confidence intervals of the median. Differences in expression levels were tested for statistical significance using the non-parametric Mann–Whitney *U*-test. $^{*}P<0{\cdot}05$; $^{**}P<0{\cdot}005$; $^{***}P<0{\cdot}001$. (a) ▲, $CD38^+DR^-$; ●, $CD38^+DR^+$; ▼, $CD38^-DR^+$. (b) ○, $DR^+CD45RO^+$; ●, $DR^+CD45RO^-$. (c) ○, $CD38^+CD45RO^+$; ●, $CD38^+CD45RO^-$.

HIV⁻ control | Asymp. HIV-1+ | Symp. HIV-1+ | AIDS HIV-1+

HLA-DR / CD45RO

CD38 / CD45RO

CD38 / HLA-DR

Fig. 3. Qualitative phenotypic changes on $CD4^+$ T cells during HIV-1 infection. The relative increase of HLA-DR and CD38 co-expression with disease progression (bottow row) is confined to $CD4^+$ T cells expressing the CD45RO.

The increasing degree of CD4+ T cell activation on one hand and the correlation between the activation of CD4+ and CD8+ T cells on the other, suggest that the progressive proportional increase of HLA-DR and CD38 expression on CD4+ T cells co-expressing CD45RO reflects an increasing state of immune activation with disease progression. In this respect, our findings support the hypothesis of AIDS as immune system activation [17].

The proportion of CD4+ T cells with the CD38+HLA-DR+, CD38+CD45RO+ and HLA-DR+CD45RO+ phenotype did not differ significantly in subjects from the various symptomatic disease stages (WHO 2 and 3 *versus* WHO 4). This lack of difference could have been biased by the clinical classification itself. When these CD4+ T cell activation markers in symptomatic subjects were calculated and classified according to decreasing CD4+ T cell percentage intervals, a clear increase was observed with decreasing CD4 percentages (data not shown).

No selective loss of CD45RO+ or CD45RO− cells within the CD4+ T cell subset was observed with disease progression, which is in agreement with previous studies [7–10]. In this regard, the preferential infection of memory CD4+ T cells by HIV-1 [18] and the finding that activated but not necessarily infected CD4+ lymphocytes from HIV-1-infected subjects are the target of autoreactive cytotoxic T lymphocytes [10,19] are not reflected in a selective depletion of CD45RO+ CD4+ T cells. Nevertheless, the CD45RO+ subset of CD4 lymphocytes displays predominant proliferative defects and is several times more vulnerable to activation-associated lymphocyte death than the CD45RO− subset [20]. This paradox might be explained by a dynamic equilibrium in which the spontaneously dying or actively destroyed CD45RO+CD4+ T cells are replaced by other CD4+ T cells, continuously being switched from CD45RA+ to CD45RO+ by non-specific activation [21]. Although reconciling seemingly contradictory data, our hypothesis is of course difficult to prove *in vivo*.

The present study showed that the relative expression of HLA-DR and CD38 on CD4+ T cells is increased in HIV-positive subjects and becomes relatively more important with progression from the asymptomatic to the symptomatic disease stage. It has been shown that anti-retroviral therapy can temporarily down-regulate the expression levels of HLA-DR and CD38 on CD8 cells [4,22]. To examine the effect of anti-retroviral treatment on the expression levels of these antigens on CD4 cells, we compared a group of treated patients with a group of untreated patients, matched for CD4 percentages and absolute numbers of CD4+ T cells; however, no significant differences could be demonstrated. To explore the possibility that treatment has a temporary effect on activation antigens on CD4 cells as previously shown for CD8 cells, a longitudinal study would be needed.

In conclusion, this study showed that the expression of activation antigens CD38 and HLA-DR within the CD4+ T cell subset changed significantly after HIV infection and increased with disease progression. A significant correlation was observed between activation of CD4+ and CD8+ T cells on one hand and between activation of CD4+ T cells and the presence of HIV antigen on the other. The usefulness of assessing these two activation antigens on CD4+ T cells as surrogate markers for therapy or as prognostic markers for disease progression remains to be determined.

ACKNOWLEDGMENTS

The authors wish to thank Dr Ludo Muylle from the Antwerp Blood Transfusion Centre for providing the control blood samples, and Ms Greet Verhulst for secretarial assistance.

REFERENCES

1 Fahey JL, Taylor JMG, Detels R *et al.* The prognostic value of cellular and serologic markers in infection with human immunodeficiency virus type 1. N Engl J Med 1990; **322**:166–72.
2 Prince HE, Jensen ER. Three-color cytofluorometric analysis of CD8 cell subsets in HIV-1 infection. J Acquir Immun Defic Syndr 1991; **4**:1227–32.
3 Kestens L, Vanham G, Gigase P *et al.* Expression of activation antigens, HLA-DR and CD38 on CD8 lymphocytes during HIV-1 infection. AIDS 1992; **6**:793–7.
4 Levacher M, Hulstaert F, Tallet S, Ullery S, Pocidalo JJ, Bach BA. The significance of activation markers on lymphocytes-CD8 in human immunodeficiency syndrome—staging and prognostic value. Clin Exp Immunol 1992; **90**:376–82.
5 Prince HE, York J, Jensen ER. Phenotypic comparison of the three populations of human lymphocytes defined by CD45RO and CD45RA expression. Cell Immunol 1992; **145**:254–62.
6 Jackson DG, Bell JI. Isolation of a cDNA encoding the human CD38 (T10) molecule, a cell surface glycoprotein with an unusual discontinuous pattern of expression during lymphocyte differentiation. J Immunol 1990; **144**:2811–5.
7 Vuillier F, Lapresle C, Dighiero G. Comparative analysis of CD4-4B4 and CD4-2H4 lymphocyte subpopulations in HIV negative homosexual, HIV seropositive and healthy subjects. Clin Exp Immunol 1988; **71**:8–12.
8 Prince HE, Czaplicki CD. Preferential loss of Leu-8−, CD45R−, HLA-DR+ CD8 cell subsets during *in vitro* culture of mononuclear cells from human immunodeficiency virus type-1 (HIV)-seropositive former blood donors. J Clin Immunol 1989; **9**:421–8.
9 Reddy MM, Grieco MH. Quantitative changes in T-helper inducer (CD4+ CD45RA−), T-suppressor inducer (CD4+ CD45RA+), T-suppressor (CD8+ CD11b+), and T-cytotoxic (CD8+ CD11b−) subsets in human immunodeficiency virus infection. J Clin Lab Anal 1991; **5**:96–100.
10 Vanham G, Kestens L, Penne G *et al.* Subset markers of CD8+ cells and their relation to enhanced cytotoxic T-cell activity during human immunodeficiency virus infection. J Clin Immunol 1991; **11**:345–56.
11 Osmond DH, Shiboski S, Bacchetti P, Winger EE, Moss AR. Immune activation markers and AIDS prognosis. AIDS 1991; **5**:505–11.
12 WHO. Acquired immunodeficiency syndrome (AIDS). Interim proposal for a WHO staging system for HIV infection and disease. Week Epidem Rec 1990; **65**:221–8.
13 Nishanian P, Huskins KR, Stehn S, Detels R, Fahey JL. A simple method for improved assay demonstrates that HIV p24 antigen is present as immune complexes in most sera from HIV-infected individuals. J Infect Dis 1990; **162**:21–28.
14 Kestens L, Hoofd G, Gigase PL, Deleys R, van der Groen G. HIV antigen detection in circulating immune complexes. J Virol Methods 1991; **31**:67–76.
15 Velleman PF, Hoaglin DC. Applications, basics and computing of exploratory data analysis. Boston: Duxburry Press, 1981.
16 Zola H, Flego L, Macardle PJ, Donohoe PJ, Ranford J, Roberton D. The CD45RO (p180, UCHL1) marker: complexity of expression in peripheral blood. Cell Immunol 1992; **145**:175–86.
17 Ascher MS, Sheppard HW. AIDS as immune system activation. 2. The panergic imnesia hypothesis. J Acquir Immun Defic Syndr 1990; **3**:177–91.

18 Schnittman SM, Greenhouse JJ, Psallidopoulos MC *et al.* Increasing viral burden in CD4+ T cells from patients with human immunodeficiency virus (HIV) infection reflects rapidly progressive immunosuppression and clinical disease. Ann Intern Med 1990; **112**:438–43.
19 Zarling JM, Ledbetter JA, Sias J *et al.* HIV-infected humans, but not chimpanzees, have circulating cytotoxic T lymphocytes that lyse uninfected CD4+ cells. J Immunol 1990; **144**:2992–8.
20 Janossy G, Borthwick N, Lomnitzer R *et al.* Lymphocyte activation in HIV-1 infection. 1. Predominant proliferative defects among CD45RO+ cells of the CD4 and CD8 lineages. AIDS 1993; **7**: 613–24.
21 Pinto L, Covas MJ, Victorino RMM. Loss of CD45RA and gain of CD45RO after *in vitro* activation of lymphocytes from HIV-infected patients. Immunology 1991; **73**:147–50.
22 Bass HZ, Hardy WD, Mitsuyasu RT, Wang YX, Cumberland W, Fahey JL. Eleven lymphoid phenotypic markers in HIV infection: selective changes induced by Zidovudine treatment. J Acquir Immun Defic Syndr 1992; **5**:890–7.