Clin. exp. Immunol. (1988) **73**, 1-5

Serum HIV antigen and anti-P24-antibodies in 200 HIV seropositive patients: correlation with CD4 and CD8 lymphocyte subsets

J.-M. ANDRIEU, DENISE EME, A. VENET, CHRISTINE AUDROIN, J.-M. TOURANI, M. STERN, DOMINIQUE ISRAEL-BIET, KHEIRA BELDJORD, FRANÇOISE DRISS & PHILIPPE EVEN
Laennec HIV Study Group, Hôpital Laennec, Paris, France

(Accepted for publication 16 February 1988)

SUMMARY

Serum HIV (P24) antigen (Ag) measured by an antigen capture ELISA (Abbott) and anti-P24-antibodies (Abs) measured by a competitive ELISA (Abbott) and by Western Blot (Dupont de Nemours) analysis were correlated with lymphocyte subsets (CD4 and CD8) in 174 HIV seropositive patients without AIDS (non-AIDS) and 26 with AIDS. In the non-AIDS group, 27% of the patients were anti-P24-Ab negative and 21% were Ag positive while in the AIDS group these figures were 62% and 54% respectively ($P < 0{\cdot}001$). Overall, a significant correlation exists between the Ab–Ag profile and the CD4 cell count: the percentage of patients with anti-P24-Ab positive and Ag negative decreases from 90% for patients with more than 900 CD4 cells/μl to 21% for patients with 100 and less CD4 cells/μl; on the contrary, the percentage of patients with anti-P24-Ab negative and Ag positive increases from 0% over 800 CD4 cells to 53% under 100 CD4 cells/μl. A weak correlation may also exist with the CD8 cell count. The subgroup of patients with 1,000 or more CD8 cells/μl have a higher (but not significant) percentage of subjects with Ag positive and anti-P24-Ab negative than the subgroup with less than 1,000 CD8 cells/μl. A short-term longitudinal study (mean follow-up: 1 year) was performed on 80 non-AIDS subjects: 77% (10/13) of those who had a CD4 cell decrease (> 30%) were initially Ag positive, while only 21% (14/67) of those without a decrease were Ag positive at the beginning ($P < 0{\cdot}1$). Although the relative weight and individual predictive value of each of these parameters need to be classified, they are probably the best biological markers currently available for monitoring clinical trials with experimental drugs.

Keywords AIDS P24 antigen anti-P24-antibodies T cell subsets

INTRODUCTION

Through a mechanism which has not been clearly elucidated yet, human immunodeficiency virus (HIV) infection produces a progressive CD4 cell decrease in most HIV seropositive subjects (Fahey *et al.*, 1987). Acquired immunodeficiency syndrome (AIDS) occurs generally when the CD4 cell count is markedly reduced and it has now been demonstrated in several cohorts that there is a high correlation between the CD4 cell count and the risk of AIDS. In addition, the development of AIDS has recently been shown to be frequently associated with the decline in the antibody level against the major HIV core protein P24 (Barin *et al.*, 1985; Biggar *et al.*, 1985; Franchini *et al.*, 1987; Weber *et al.*, 1987) and with the appearance of HIV (P24) antigen in serum (Goudsmit *et al.*, 1986a; Lange *et al.* 1986).

The aim of this study is to correlate the serum HIV antigen and anti-P24-antibody patterns with CD4 and CD8 cell subsets in 174 HIV seropositive patients without AIDS and 26 with AIDS.

Correspondence: Professor Jean-Marie Andrieu, Oncology/Hematology, Laennec Hospital, 42 rue de Sèvres, 75007 Paris, France.

MATERIALS AND METHODS

Between October 1985 and December 1986, 174 HIV seropositive subjects without AIDS (non-AIDS) and 26 patients with AIDS were referred to our Laennec HIV study group. After complete clinical examination and HIV seropositivity confirmation, blood counts and CD4 cell subset measurements were performed at least twice within 1–8 weeks in all non-AIDS patients while CD8 cells were measured in 101 of them. CD4 and CD8 cell measurements were performed after Ficoll-Hypaque sedimentation by indirect immunofluorescence using anti-CD4 and anti-CD8 monoclonal antibodies (MoAb).

Non-AIDS subjects were classified according to our Laennec working staging system (LWSS) (Andrieu, Even & Venet, 1986). Three stages (CD4 stages) were determined according to the mean CD4 cell count : CD4 stage I over 600 CD4 cells/μl, CD4 stage II between 300 and 600 CD4/μl, CD4 stage III and under 300 CD4/μl. Three clinical classes were also identified according to the clinical status of the patients: A, asymptomatic subjects; B, presence of persistent generalized lymphadenopathies (PGL) without any symptoms; C, presence of at least one of

Table 1. Classification of the 174 non-AIDS and 26 AIDS patients in the Laennec working staging system

	Clinical classes						
	Non-AIDS			AIDS*			
CD4 stage	A	B	C	I	K	E	L
I	35	34	2	1	—	—	—
II	31	35	6	1	1	—	—
III	7	12	12	14	7	1	1

Stage I, CD4 > 600/μl; Stage II, CD4 between 300 and 600/μl; Stage III, CD4 < 300/μl; A, asymptomatic; B, persistent generalized lymphadenopathies; C, constitutional symptoms; I, opportunistic infection; K, Kaposi's sarcoma; E, encephalopathy; L, Non-Hodgkin lymphoma.

* Only the first episode of AIDS is taken into account.

Table 2. Anti-P24-antibody (Ab) and HIV antigen (Ag) patterns of 174 non-AIDS and 26 AIDS classified according to their clinical status

		Ab–Ag status (percent of patients)			
Clinical classes of HIV disease	Patient number	Ab pos and Ag neg	Ab neg and Ag neg	Ab pos and Ag pos	Ab neg and Ag pos
Non-AIDS	174	70	9	3	18
A	73	78	4	3	15
B	81	68	11	4	17
C	20	50	15	0	35
AIDS	26	30	16	8	46

Table 3. Anti-P24-antibody (Ab) and HIV antigen (Ag) patterns of 174 non-AIDS patients classified according to their CD4 cell count

		Ab–Ag status (percent of patients)			
CD4 stages	Patient number	Ab pos and Ag neg	Ab neg and Ag neg	Ab pos and Ag pos	Ab neg and Ag pos
I	71	83	8	3	6
I/II, *P**		< 0·02	NS	NS	= 0·001
II	72	64	8	1	27
II/III, *P**		NS	NS	NS	NS
III	31	55	10	6	29

* Chi-Square test.

CD4 stage: I, CD4 cells/μl > 600; II, CD4 cells > 300 and < 600; III, CD4 cells/μl < 300.

the following symptoms with or without PGL: fever over 38°C, night sweating, weight loss over 10% diarrhoea of three or more stools per day, oral thrush. Clinical class A corresponds to CDC classification group II, class B to group III and class C to group IV, subgroup A (symptoms) and C2 (thrush).

Ten millilitres of serum from every patient was frozen at −20°C. Between March and June 1987, serum samples were assayed for the presence or absence of HIV (P24) antigen (Ag) with an antigen capture ELISA (Abbott Kit) using polyclonal antibodies to HIV-1 as capture and probe antibodies (Goudsmit *et al.*, 1986a). Specificity of positive results was checked by a neutralizing test using human polyclonal antibodies to HIV-1. The presence or absence of anti-P24 antibodies (Ab) was checked by a competitive ELISA (Envacore Abbott) with recombinant antigen containing all p24 and part of p15 and p17 gag antigens (Goudsmit *et al.*, 1986b). A Western blot (WB) (Dupont de Nemours) was also performed in all patients for comparison with ELISA data. The anti P24-Ab status with WB was evaluated as negative, weakly positive or positive.

The Chi-square test was used to analyse the results.

RESULTS

Initial characteristics

The demographic chracteristics of the 200 patients were: 176 male, 24 female; age, 19–26 years, median 31; mode of HIV transmission, 152 by sexual contact, 47 by sharing syringes, 1 by blood transfusion.

The distribution in the LWSS of the 26 AIDS and 174 non-AIDS patients is given in Table 1.

Clinical status

Table 2 gives the Ab–Ag status of the 26 AIDS and 174 non-AIDS subjects. In the non-AIDS group 27% of the subjects were anti-P24-Ab negative (neg.) and 21% were Ag positive (pos.) while these percentages increased in the AIDS group to 62%

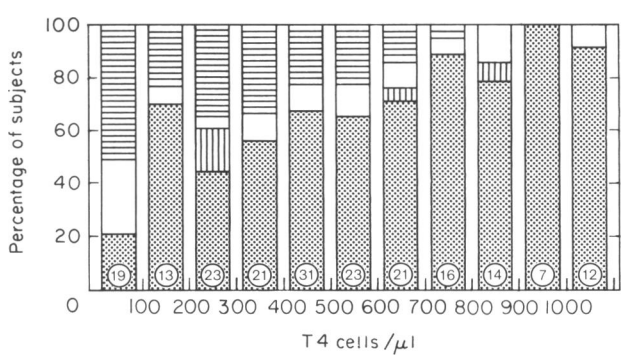

Fig. 1. Correlation between CD4 cell count and anti-P24-antibody (Ab)-HIV antigen (Ag) status (▨, Ab+Ag −; ■, Ab−Ag+; ■, Ab+Ag+; □, Ab−Ag−). The number of subjects in each hundredth of CD4 cells is given in the open circle at the bottom of each column.

Table 4. Anti-P24 antibody (Ab) and HIV antigen (Ag) patterns of 101 patients classified according to their CD8 cell count

CD4 stage	CD8 cell count/μl	Patient number	Ab–Ag status (percent of patients) Ab pos and Ag neg	Ab neg and Ag neg	Ab neg and Ag pos	Ab pos and Ag pos
I	<1,000	21	95	5	0	0
	>1,000	17	82	6	6	6
II	<1,000	31	68	6	26	0
	>1,000	14	36	14	36	14
III	<1,000	13	54	8	30	8
	>1,000	5	40	0	60	0
All	<1,000	65	74	6	19	1
	>1,000	36	59	8	25	8

Table 5. Correlation between initial HIV (P24) antigenaemia and CD4 cell count modifications after 12+5 months of follow-up

Initial HIV (P24) antigenemia	One-year CD4 cell count decrease <30%	>30%
Negative	53	3
Positive	14	10
All	67	13

($P<0.001$) and 54% ($P<0.001$) respectively. On the other hand, the percentage of subjects with both anti-P24-Ab neg. and Ag pos. increased from 18% in the non-AIDS group to 46% in the AIDS group ($P<0.01$). It is noteworthy that 30% of the patients with AIDS were anti-P24-Ab pos.-Ag neg.

Inside the non-AIDS group, subjects belonging to clinical classes A and B had the same low percentage of individuals with both anti-P24-Ab neg. and Ag pos. (15% and 17% respectively). This percentage increased to 35% in patients of clinical class C (P=NS) (Table 2).

Immunological status

CD4 cell count. There is a relation between the CD4 cell count and the Ag–Ab status. Inside the non-AIDS group, the percentage of subjects with anti-P24-Ab pos. and Ag neg. decreases from stage I to stage III while the percentage of patients with anti-P24-Ab neg. and Ag pos. increases (Table 3). Figure 1 gives a more precise picture of this correlation on the whole group of patients (AIDS and non-AIDS). It shows the percentages of patients with the different serum Ab-Ag patterns for each hundredth of CD4 cells. The percentage of patients with anti-P24-Ab pos. and Ag neg. decreases from 90% for patients with more than 900 CD4 cells/μl to 21% for patients with less than/μl 100 CD4. On the contrary, the percentage of patients with anti-P24-Ab neg. and Ag pos. increases from 0% over 800 CD4 cells to 53% under 100 CD4 cells.

CD8 cell count. Both CD4 and CD8 cells were simultaneously measured in 101 non-AIDS subjects. Table 4 shows that for each CD4 stage, the subset of patients with more than 1,000 CD8 cells/μl has a higher probability of being anti-P24 neg. Ag pos. than the subset with less than 1,000 CD8 cells/μl: stage I 6% VS 0%, stage II 36% VS 26%, stage III 60% VS 30%. Conversely, the probability of being anti-P24-Ab pos. and Ag neg. is lower in patients with less than 1,000 CD8 cells/μl than in those with more than 1000 CD8 cells/μl : stage I 82% VS 95%, stage II 36% VS 68%, stage III 40% VS 54%.

Comparison of anti-P24 antibodies by ELISA and western-blot

WB and ELISA methods were compared for the presence of anti-P24-Ab in the serum of the 200 patients. All 137 patients who were positive with ELISA were also positive with WB. Out of the 63 patients whose ELISA was negative 10, 23 and 30 were respectively positive, weakly positive and negative with WB. Overall, the percentage of subjects with negative or weakly positive WB for anti-P24-Ab was higher in stage III (25/54) than in stage II (23/74) ($P>0.05$) and significantly higher in stage II than in stage I (8/72) ($P=0.006$).

Short term follow-up of HIV antigenaemia and CD4 cell counts

Eighty non-AIDS subjects were followed-up for 4 to 21 months (mean 12±5). Fifty-six of them were initially Ag neg. At the end of the follow-up period, 52 remained neg., and four became Ag pos. at a low serum concentration (50–120 pg/ml). The other 24 subjects were initially Ag pos. with a mean serum concentration of 216 pg/ml (±222). Twenty remained positive and four became negative. Overall the mean concentration of these 24 subjects had increased to 1033 pg/ml (±3430) ($P<0.02$). Over this short period of follow-up, a correlation was observed between initial Ag status and the evolution of the CD4 cell count as shown in Table 5. Out of the 24 subjects initially Ag positive, 10 (42%) had presented a CD4 cell count decrease greater than 30% (three measurements or more within the last 2 months of follow-up) whereas out of the 56 subjects initially Ag neg. only three had such a decrease (5·4%, $P<0.01$).

DISCUSSION

This study provides information on the serological patterns (HIV P24-Ag and anti-P24-Ab) of 174 non-AIDS and 26 AIDS patients staged according to their clinical as well as immunological (CD4 and CD8 subsets) status. The proportion of subjects with positive Ag is significantly higher in AIDS (54%) than in non-AIDS subjects (21%). This confirms several transversal studies (Goudsmit *et al.*, 1986a,b; Lange & Goudsmit, 1987) and longitudinal studies (Biggar *et al.*, 1985; Lange *et al.*, 1986; Weber *et al.*, 1987; Kenny *et al.*, 1987) recently performed on small cohorts of patients. The proportion of subjects without anti-P24-Ab is also significantly higher in AIDS (62%) than in non-AIDS (27%).

Inside the non-AIDS group this study demonstrates that the proportion of subjects with positve Ag is low in clinical classes A and B. It increases in class C, but not statistically (probably because of the small number of patients). On the other hand a significant correlation exists with the CD4 cell status: the lower the CD4 cell count, the higher the proportion of subjects with

positive Ag (Table 3 and Fig. 1). A similar correlation is observed for the proportion of subjects without anti P24-Ab, both with ELISA (Table III) and with the much more sensitive technique of WB.

The serological patterns of this large group of non-AIDS subjects and the probability of HIV seropositive subjects acquiring AIDS (as recently evaluated in longitudinal studies by several groups of investigators) can be correlated. The groups of patients without symptoms or with lymphadenopathies alone (clinical classes A and B) have the same low percentage of individuals with Ag pos. and anti-P24-Ab neg. (Table 2). They are also known to have the same low probability of acquiring AIDS (Polk *et al.*, 1987). The group of patients suffering from constitutional symptoms (clinical class C) has a higher percentage of subjects with Ag pos. and Ab neg. (Table 2); they are also at high risk of AIDS development within a given time (Polk *et al.*, 1987).

When looking at the CD4 cell count, the correlation between our results and longitudinal studies performed by others is more obvious. The lower the CD4 cell count, the higher the percentage of subjects with Ag pos. (Fig. 1 and Table 3) and the higher the risk of AIDS (Fahey *et al.*, 1987; Polk *et al.*, 1987). These data emphasize the need for classifications that take into account the immunological status. Such staging systems like our LWSS or the Walter Reed classification appear more relevant than the CDC classification.

The short term longitudinal study that we performed on 80 subjects (mean follow-up 12 ± 15 months) reinforced these results: 77% (10/13) of the subjects who had a CD4 cell decrease (> 30%) were Ag pos. at the beginning of the follow-up period whereas only 21% (14/67) without a CD4 cell decrease were also Ag pos at that time ($P < 0{\cdot}01$, Table 5). In Ag pos. subjects, the concentration of Ag increases with time. The same phenomenon was recently observed in the placebo arm of the azydothymidine clinical trial (Chaisson *et al.*, 1986). However the Ag increase was not statistically significant, probably as a consequence of the short period of follow-up of the two studies (12 and 6 months respectively).

A correlation between the CD8 cell count, Ab and Ag status and the probability of AIDS may also exist. The percentage of patients with Ab neg and Ag pos. is higher (however not significantly) in the subset of subjects with more than 1,000 CD8 cells/μl than in the group of patients with less than 1,000 CD8 cells/μl (Table 4) and the risk of AIDS has been recently recognized as higher over 1,000 CD8 cell/μl than under this count (Polk *et al.*, 1987).

It is difficult to appraise the significance of anti-P24-Ab neg-Ag neg. and anti-P24-Ab pos-Ag pos. profiles. (HIV) Ag ELISA is not specific for P24 and quantifies other HIV proteins also. The ELISA anti P24-antibody method recognizes antibodies against other Gag products such as P15 and P17. Moreover, specific anti-P24-antibodies continue to be detectable in some cases by WB when the ELISA test is negative. Although the number of subjects having anti-P24-Ab pos Ag pos or anti-P24-Ab neg Ag neg patterns is small, it is likely that they are more frequent in patients with AIDS and/or with low CD4 cell count than in HIV seropositive patients without AIDS and/or higher CD4 cell count (Table 2 and Fig. 1).

The relation linking anti-P24-Ab decrease, serum HIV Ag increase and CD4 cell decrease remains to be elucidated. *In vivo*, the respective roles of CD4 cells, monocytes and Langerhans cells in the production of serum HIV Ag is not known so far (Gartner *et al.*, 1986). Free anti-P24-Ab disappearance may partly be the consequence of the increase of HIV Ag synthesis (Lange & Goudsmit, 1987) and release through the formation of immune complexes (Frosner *et al.*, 1987). It may also be the consequence of the destruction of anti-P24-antibody forming cells and/or the decrease of CD4 cell help for antibody synthesis. However, in Africa, when tested by the same anti-P24 ELISA kit (Abbott) more than 90% of all HIV-infected subjects remain positive (Barin, 1987) even when they have acquired AIDS. As far as we know, there is no information on the HIV antigenaemia of these African subjects. In any case, this observation renders unlikely the possibility that an anti-P24-Ab stimulation by vaccination could delay or prevent AIDS occurrence in HIV-seropositive persons as it was recently proposed (Salk, 1987).

Overall, this transectional study indicates that in European seropositive subjects without AIDS the presence of HIV Ag in serum (ELISA) and the decrease or disappearance of anti-P24-Ab are correlated with the decrease of the CD4 cell count of these subjects. A correlation between Ag–Ab status and the CD8 cell count ($< 1{,}000/\mu$l VS $> 1{,}000/\mu$l) may also exist.

We are now engaged in the long-term follow-up of this cohort of non-AIDS subjects to confirm these correlations on a longitudinal basis and to find out the relative weight and the individual predictive value of each of these parameters. HIV Ag-anti-P24-Ab status should also be correlated with genetic variables such as HLA which has recently been claimed of prognosis interest (Scorza Smeraldi *et al.*, 1986). These parameters may also serve for monitoring clinical trials with experimental drugs.

REFERENCES

Andrieu, J.M., Even, P. & Venet, A. (1986) AIDS and related syndromes as a virus-induced autoimmune disease of the immune system: an anti-MHC II disorder. Therapeutic implications. *AIDS Research* **2**, 163.

Barin, F. (1987) AIDS vaccine predictions. *Nature* **328**, 21.

Barrin, F., McLane, J., Allan, L.S., Lee, T.H., Groopman, J.E. & Essex, M. (1985) Virus envelope protein of HTLV III represents major antigen for antibodies in AIDS patients. *Science* **228**, 1094.

Biggar, R.J., Melbye, M., Ebbesen, P., Alexander, S., Nielsen, J.O., Sarin, P. & Faber V. (1985) Variation in human T lymphotropic virus III (HTLV-III) antibodies in homosexual men: decline before onset of illness related to acquired immune deficiency syndrome (AIDS). *Br. med. J.* **291**, 997.

Chaisson, R., Allain, J.P., Leuther, H. & Volberding, P. (1986) Significant changes in HIV antigen level in the serum of patients treated with azydothymidine. *N. Engl. J. Med.* **315**, 1610.

Fahey, J.L., Giorgi, J., Martinez-Maza, O., Detels, R., Mitsuyasu, R. & Taylor J. (1987) Immune pathogensis of AIDS and related syndromes. In *Acquired Immunodeficiency Syndrome* (eds J.C. Gluckman & E. Vilmer) p 107. Elsevier, Paris.

Franchini, G., Robert-Guroff, M., Aldovini, A.A., Kan, N.C. & Wong-Staal, F. (1987) Spectrum of natural antibodies against five HTLV-III antigens in infected individuals : correlation of antibody prevalence with clinical status. *Blood* **69**, 437.

Frosner, G.G., Effle, V., Mellert, W. & Hehlmann, R. (1987) Diagnostic significance of quantitative determination of HIV antibody specific for envelope and core proteins. *Lancet* **i**, 159.

Gartner, S., Markovits, P., Markovitz, D.M., Kaplan, M.H., Gallo, R.C. & Popovic, M. (1986) The role of mononuclear phagocytes in HTLV-III/LAV infection. *Science* **233**, 215.

GOUDSMIT, J., DE WOLF, F., PAUL, D.A., EPSTEIN, L.G., LANGE, J.M.A., KRONE, W.J.A., SPEELMAN, H., WOLTERS, E. CH., VAN DER NORDAA, J., OLESKE, J.M., VAN DER HELM, J. & COUTINHO, R.A. (1986a) Expression of human immunodeficiency virus antigen (HIV-Ag) in serum and cerebrospinal fluid during acute and chronic infection. *Lancet* **ii,** 177.

GOUDSMIT, J., LANGE, J.M., PAUL, D.A. & DAWSON, G.J. (1986b) Antigenemia and antibody titers to core and envelope antigens in AIDS, AIDS-related complex, and subclinical human immunodeficiency virus infection. *J. infect. Dis.* **155,** 558.

KENNY, C., PARKIN, J., UNDERHILL, G., SHAH, N., BURNELL, B., OSBORNE, E. & JEFFRIES, D.J. (1987) HIV antigen testing. *Lancet* **i,** 565.

LANGE, J.M., PAUL, D.A., HUISMAN, H.G., DE WOLF, F., VAN DEN BERG, H., COUTINHO, R.A., DANNER, S.A., VAN DER NOORDAA, J. & GOUDSMIT, J. (1986) Persistent HIV antigenaemia and decline of HIV core antibodies associated with transition to AIDS. *Br. med. J.* **293,** 1459.

LANGE, J.M. & GOUDSMIT, J. (1987) Decline to antibody reactivity to HIV core protein secondary to increased production of HIV antigen. *Lancet* **i,** 448.

POLK, B.V., FOX, R., BROOKMEYER, R., KANCHANARAKSA, S., KASLOW, R., VISSCHER, B., RINALDO. C. & PHAIR, J. (1987) Predictors of the acquired immunodeficiency syndrome developing in a cohort of seropositive homosexual men. *N. Engl. J. Med.* **316,** 61.

SALK, J. (1987) Prospects for the control of AIDS by immunizing seropositive individuals. *Nature* **327,** 473.

SCORZA SMERALDI, R., FABIO, G., LAZZARIN, A., EISERA, N.B., MORONI, M. & ZANUSSI, C. (1986) HLA associated susceptibility to acquired immunodeficiency syndrome in Italian patients with human-immunodeficiency-virus infection. *Lancet* **ii,** 1187.

WEBER, J.N., CLAPHAM, P.R., WEISS, R.A., PARKER, D., ROBERTS, C., DUNCAN, J., WELLER, I., CARNE, C., TEDDER, R.S., PINCHING, A.J. & CHEINGSONG-POPOV, R. (1987) Human immunodeficiency virus infection in two cohorts of homosexual men: neutralising sera and association of anti-gag antibody with prognosis. *Lancet* **i,** 119.