

Circulation of HIV antigen in blood according to stage of infection, risk group, age and geographic origin

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SUMMARY

Human immunodeficiency virus antigen (HIV-ag) was determined by enzyme immunoassay (EIA) in HIV-antibody (anti-HIV) positive as well as pre-anti-HIV seroconversion sera and the results analysed according to stage of infection, risk group, age and geographic origin. Eleven (19%) of 58 homosexual men tested showed HIV-ag in a serum taken 3–4 months before or one at the time of anti-HIV seroconversion. In another eight (14%) HIV-ag persisted after seroconversion and half of them developed AIDS or AIDS-related complex (ARC) in contrast to none of the other 50 anti-HIV seroconversions. Two (13%) of 16 haemophiliacs tested had HIV-ag only in the first anti-HIV seropositive sample. HIV-ag was present in 86% (30/35) of Dutch homosexual men with AIDS, in 32% (7/22) of men with ARC and in 17% (24/145) of men with persistent generalized lymphadenopathy (PGL) or without symptoms. Three percent (2/60) of sera of asymptomatic i.v. drug users from Amsterdam were HIV-ag positive. Ten percent (1 of 10) of sera from Central Africans with 'Slim Disease' were HIV-ag positive. Among infected children from the USA or Europe 89–100% (8/9 and 2/2) of AIDS cases, 67–100% (6/9 and 3/3) of children with ARC and 75% (3/4) of asymptomatic children were HIV-ag positive. The HIV-ag EIA appears to be able to identify HIV infection earlier than the available anti-HIV assays in a significant number of cases. Since persistence of HIV-ag, except possibly in African cases, is strongly associated with clinical deterioration, HIV-ag appears to be a suitable marker for, independent of their clinical status, selecting individuals for antiviral therapy and also for monitoring the efficiency of such therapy.

INTRODUCTION

Human immunodeficiency virus (HIV), the causative agent of acquired immune deficiency syndrome (AIDS), can be isolated from phytohaemagglutinin-stimulated peripheral blood cells of both clinically ill and asymptomatic HIV infected individuals (Gallo, Salahuddin & Popovic, 1984; Levy & Shimabukuro, 1985). Recently an enzyme immunoassay (EIA) for the detection of HIV antigens

(HIV-ag) has been developed (Paul & Falk, 1986). HIV-ag could be detected transiently in serum before anti-HIV appeared (Goudsmit *et al.* 1986; Allain *et al.* 1986). Persistence or reappearance of HIV-ag in serum accompanied by a decline in antibody reactivity to the major core protein p 24 of HIV has been associated with AIDS and the transition to AIDS (Goudsmit *et al.* 1986; Lange *et al.* 1986a; Goudsmit *et al.* 1987). In this paper we analysed the performance of this HIV-ag EIA during acute as well as chronic HIV infections in the major risk groups, i.e. homosexual men, haemophiliacs, i.v. drug users, children from Europe and the USA as well as Central Africans.

MATERIAL AND METHODS

Serum samples

Sera originated from several sources. The first group consisted of homosexual men from Amsterdam. Thirty-five were patients with AIDS (CDC IV C & D) (CDC, 1986), who were either participants in a prospective study on the prevalence or incidence of HIV infection or patients treated at the Internal Medicine Department of the Academic Medical Center of the University of Amsterdam. In addition serum from 22 anti-HIV seropositive men staged as CDC IV A and 145 as CDC II or III from the same sources were included. Sequential serum samples of six of these men with AIDS and six without AIDS were available for testing. Sequential sera of 58 men participating in our prospective trial and seroconverting for anti-HIV were also studied over a period of 2 years. The sera were collected approximately every 3 months. Two developed AIDS and two constitutional disease within the study period. The second group consisted of 16 haemophiliacs from the Netherlands seroconverting for anti-HIV and followed for 3 years. Sera were collected every 6 months. None of them progressed beyond persistent generalized lymphadenopathy (CDC II & III).

The third group consisted of 60 anti-HIV seropositive intravenous drug users participating in a prospective study in Amsterdam.

The fourth group consisted of 9 children with AIDS, and 9 with ARC treated at the paediatrics department of the University of Medicine and Dentistry of New Jersey, Newark, NJ, USA and 9 children (2 AIDS, 3 ARC and 4 other), treated at the paediatrics department of the Academic Hospital of Leiden University, the Netherlands.

The fifth and last group included 10 cases of 'Slim Disease' from Uganda, Central Africa and 2 anti-HIV seropositive and 20 seronegative controls from the same geographic area. Sera were stored at -20°C before testing. All sera were tested for anti-HIV with a commercially available EIA (Abbott Labs, N. Chicago, Ill., USA) and positive samples were confirmed by immunoblotting (Lange *et al.* 1986b).

HIV antigen EIA

Serum samples were assayed for HIV antigen in a solid phase immunoassay (Abbott Laboratories, North Chicago, Ill. (Paul & Falk, 1986; Goudsmit *et al.* 1986; Lange *et al.* 1986a). Two hundred microlitres of sample were incubated overnight at room temperature ($\sim 22^{\circ}\text{C}$) with a bead coated with human

antibody to HIV. Beads were washed with distilled water; rabbit IgG antibody to HIV was added and incubated for 4 h at 45 °C. Beads were washed as before and then incubated for 2 h at 45 °C with horseradish peroxidase-conjugated goat antibody to rabbit IgG. After a final wash, the beads were transferred to tubes and ortho-phenylene-diamine was added. After 30 min at room temperature in the dark, 1 ml of 1N-H₂SO₄ was added to each tube. The optical density (O.D.) of 492 nm was read by using the Quantum Dual Wavelength spectrophotometer (Abbott Laboratories). A sample was considered positive if its O.D. was ≥ 0.050 plus the mean of five duplicates of normal human plasma. The assay is most sensitive for the core antigen of HIV and detects antigen in culture supernatant of 10 cells/ml of HIV-infected HT-9 cells (uninfected HT-9 cell supernatant is negative). The specificity of the assay is determined in part by the rabbit antibody, which, when used on western blots of purified HIV lysate or in RIPA procedures, detects p55/24 (core) strongly and gp120/41 (envelope) only faintly. Purified, recombinant core antigen is detectable at 50 pg/ml when spiked into serum or plasma, whereas purified, recombinant envelope antigen is detectable at ~ 500 ng/ml. The rabbit anti-HIV used has 90% of its anti-HIV activity inhibited by HIV core protein and only 8% by HIV envelope protein. Antigen activity in serum can be neutralized 100% by pre-incubating the ag positive sample with anti-p24 from a guinea-pig immunized with purified p24. Here, quantitation of HIV antigen was done by comparing the O.D.s of the samples with the O.D.s of known quantities of purified HIV lysate.

RESULTS

Using lysate of the HIV strain HTLV-III B as reference, the sensitivity of the assay ranged from 9 to 17 pg/ml in the studies presented. The O.D. values corresponded to quantity of viral protein as illustrated in Fig. 1. HIV-ag in early HIV infection was studied in 58 homosexual men and 16 haemophiliacs, seroconverting for anti-HIV (Table 1). Sixty-seven percent of the homosexual men and 87% of the haemophiliacs did not show HIV-ag in any serum sample tested. Transient HIV-ag was found in sera taken before or at the time of anti-HIV seroconversion in 19% of the homosexual men and in 13% of the haemophiliacs tested. The earliest anti-HIV seronegative sample being HIV-ag positive was taken 4 months before anti-HIV was detected. All of these men remained without serious disease during follow-up. Serum samples of eight (14%) of the homosexual men and none of the haemophiliacs became HIV-ag seropositive at or shortly after anti-HIV seroconversion and remained HIV-ag positive during follow-up. Two of these men developed AIDS and two constitutional disease during follow-up. To illustrate the temporal relationship between the occurrence of HIV-ag and anti-HIV more accurately in this group of men, the absolute O.D. values were plotted (Fig. 2). A small rise in O.D. was observed before anti-HIV antibodies became detectable by immunoblotting, although below the cut-off value.

Two of the patients developed AIDS (Fig. 2*a, d*) and both showed a marked decrease in antibodies to the major core protein p24, while the two with constitutional disease (Fig. 2*b, c*) did not show this decrease of antibody.

Fig. 3 shows that in serum of both adults with AIDS and children with AIDS

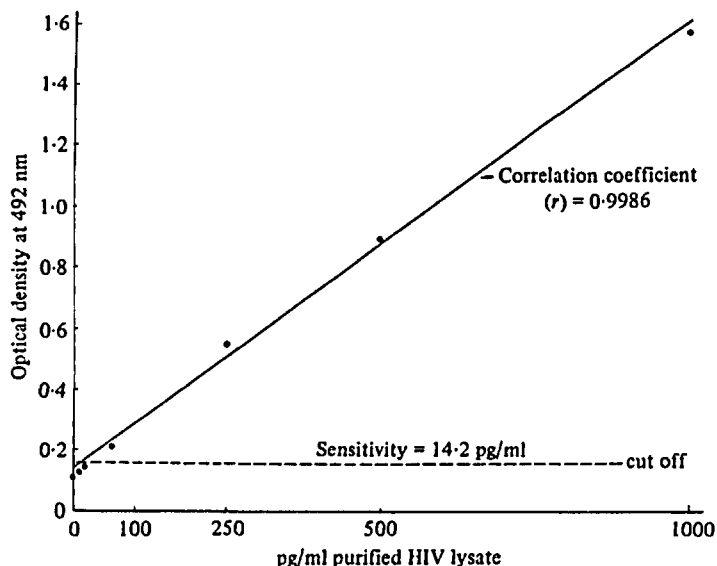


Fig. 1. Increase of o.d. values with increasing quantities of HIV antigens as evaluated with HIV lysate. Regression analysis yielded the plotted line ($r = 0.99$).

Table 1. *HIV antigen in anti-HIV seroconverting homosexual men and haemophiliacs*

Category	No. tested	HIV-ag status (%)		
		Negative	Transient positive	Persistent positive
Homosexual men	58	39(67)	11(19)	8(14)
Haemophiliacs	16	14(87)	2(13)	0

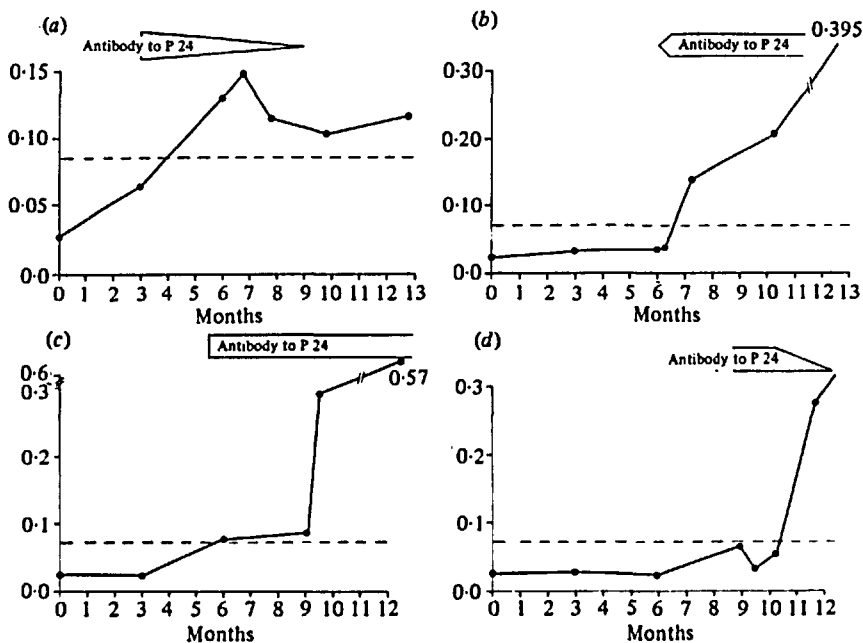


Fig. 2. Change of o.d. values in the HIV-ag EIA with sequential sera from anti-HIV seroconverted adults. All four developed AIDS or AIDS-related symptoms. (For further explanation see text.)

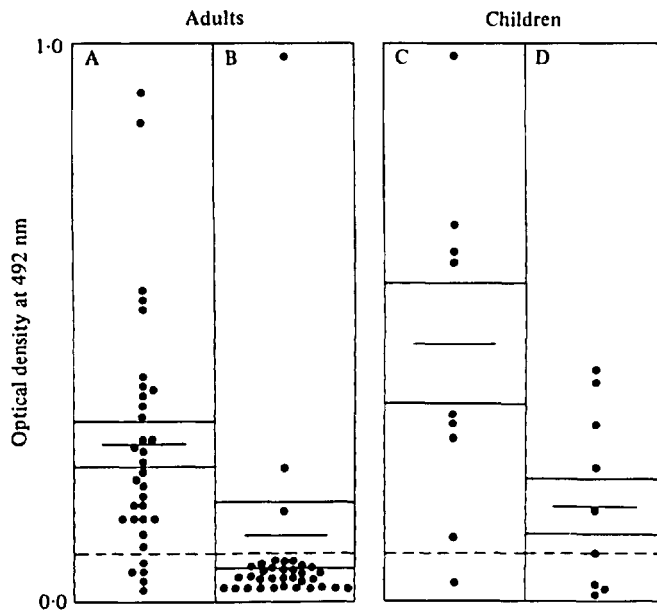


Fig. 3. Distribution of o.d. values in the HIV-ag EIA of sera from adult (panel A) and paediatric (panel C) patients with AIDS, from asymptomatic anti-HIV seropositive adults (panel B) and children with ARC (panel D). The mean o.d. (\pm S.E.M.) is indicated for each group by horizontal bars. The dashed line indicates the cut-off value (0.07).

Table 2. HIV antigen in serum of HIV antibody seropositive individuals according to risk group, geographic origin and stage of infection

Clinical category	Geographic origin	Number positive/ Number tested (%)
AIDS (CDC IV C & D)		
Homosexual men	Europe	30/35(86)
Children	USA	8/9(89)
	Europe	2/2(100)
Slim Disease	Central Africa	1/10(10)
Constitutional Disease (CDC IV-A)		
Homosexual men	Europe	7/22(32)
Children	USA	6/9(67)
	Europe	3/3(100)
Other (CDC II and III)		
Homosexual men	Europe	24/145(17)
Haemophiliacs	Europe	0/16(—)
I.v. drug users	Europe	2/60(3)
Children	Europe	3/4(75)

the reactivity in the HIV-ag EIA was separable from the reactivity in serum of adults staged as CDC II or III. However, two of the adult sera were above the cut-off value (0.07) established on anti-HIV seronegative plasma. In children without serious disease or with ARC (panel D) reactivity above the cut-off value was frequent.

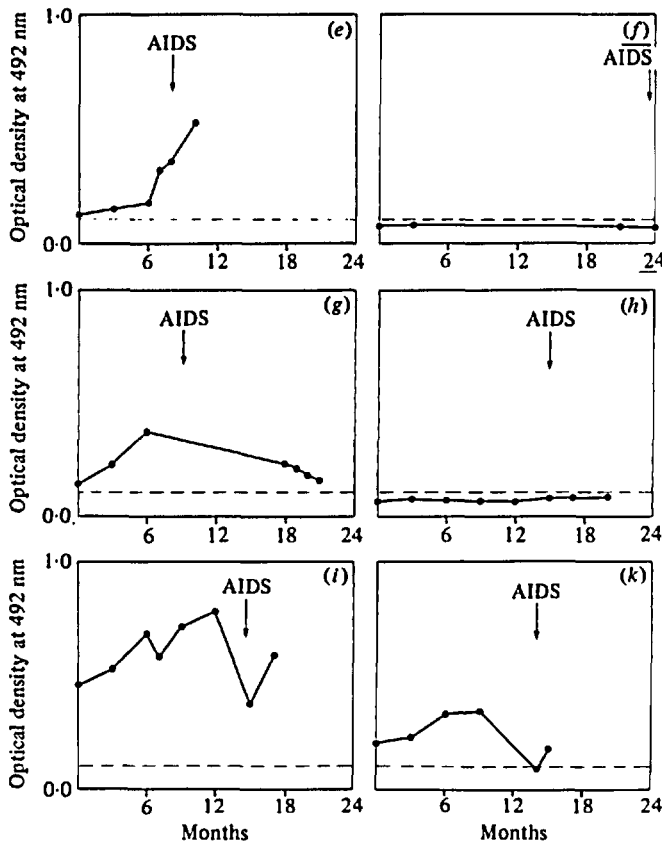


Fig. 4. Change in o.d. values in the HIV-ag EIA with sequential sera from anti-HIV seropositive adults who developed AIDS.

Table 2 depicts the prevalences of HIV-ag within different risk groups. HIV-ag was detected in serum of over 80% of adults and children with AIDS in Europe and the USA. Serum of only one Central African patient with 'Slim Disease' was positive in the HIV-ag EIA with an o.d. value close to the cut-off for the assay. Each of these sera was reactive with the gp41 transmembrane protein of the HIV strain HTLV-III and nine of the ten had antibodies to the HIV gag gene product p24. Among homosexual men with ARC 32% were HIV-ag seropositive relative to 67% among children with ARC from the USA and 100% in Europe. In the less ill individuals (CDC II & III) HIV antigenaemia in i.v. drug users and haemophiliac males was below 3% compared with 17% in homosexual men and 75% in children. Twelve patients were prospectively studied to compare the change in the o.d. values in the HIV-ag EIA of men who developed AIDS as well as patients who did not, but became HIV-ag positive (Figs. 4 and 5). In four of the AIDS patients HIV-ag became persistently detectable 6 months to a year before AIDS was diagnosed.

In one of these patients (Fig. 4e) a stable low level of HIV-ag expression is apparent during the first 6 months of follow-up. Then 2 months before AIDS was diagnosed a rapid increase of HIV-ag was recorded. In each of these patients a decline of antibodies to the major core protein p24, as demonstrated by

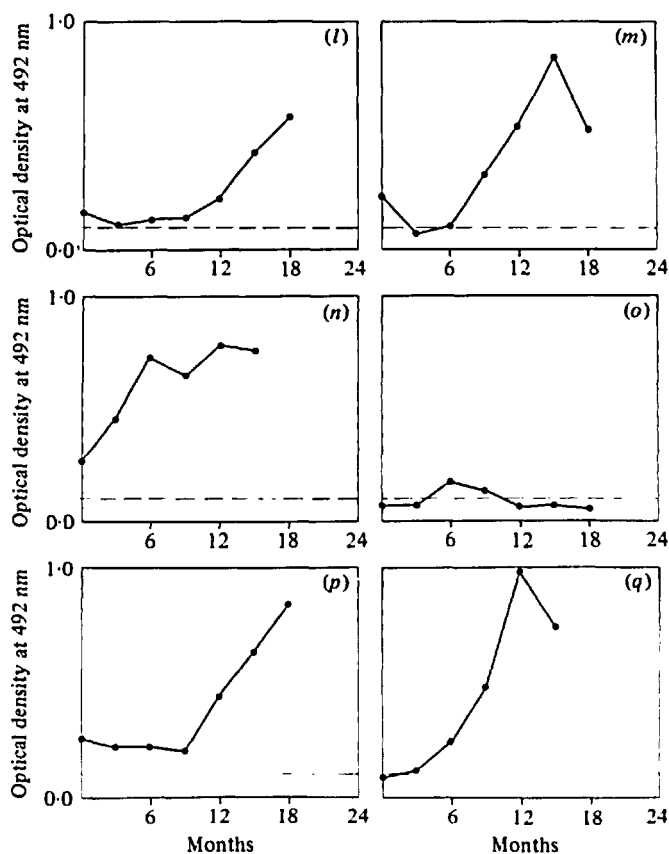


Fig. 5. Change in o.d. values in the HIV-ag EIA with sequential sera from anti-HIV seropositive adults who remained without serious clinical disease during the follow-up period.

immunoblotting accompanied the appearance of HIV antigen. Two patients (Fig. 4, *f* and *h*) showed no serum reactivity in four and eight consecutive sera before or after AIDS was diagnosed. In the first patient high levels of antibodies to p24 were demonstrated even after the diagnosis AIDS was made, in the second patient a decline of p24 antibodies was found without the subsequent detection of HIV antigen.

Five of six HIV antigenaemic men who did not develop AIDS showed persistent HIV-antigenaemia with o.d. values similar to the patients who developed AIDS (Fig. 5). Each of these cases either lacked antibodies to p24 or had declining levels of such antibodies. The sixth person (Fig. 5, *o*) showed a 3–6 months period of transient HIV antigenaemia and persistence of antibodies to p24. This pattern was only seen on three occasions among 182 men assayed and only in men without serious disease. Parts *l*, *m*, *p* & *q* of Fig. 5 show a stable low level of HIV-ag expression for a period of 6–12 months followed by a rapid increase of HIV-ag reminiscent of Fig. 4*e*.

DISCUSSION

Because the standard curve presented is based on quantities of purified HIV (strain HTLV-III B) lysate containing a mixture of viral antigens whereas the assay detects mainly the major core protein p24, the actual detection limit might be significantly lower than shown. With a recombinant p24 antigen as a standard, 10-fold less protein can be detected, indicating that approximately 10% of the purified viral lysate is composed of core protein. Specificity of the assay could be assessed two ways. We previously showed that levels of HIV-ag and HIV core antibody are balanced by the degree of viral gene expression, antibody production and immune complex formation (Lange *et al.* 1986*b*). Simultaneous testing of samples for HIV-ag and HIV core antibody will generally show high levels of one parameter in parallel with low levels of the other, in that way confirming the validity of both tests. Exceptional cases of high levels of HIV-ag in the absence of any antibody to HIV or in the presence of high levels of antibody to core need confirmation. This can be accomplished by either repeating the HIV-ag assay in the presence of a human anti-HIV serum and showing neutralization of reactivity or repeating the test altogether a few months later. Transient HIV antigenaemia before or at the time of seroconversion was observed in 13–19% of haemophiliacs and homosexual men. The earliest HIV-ag seropositive sample was taken 4 months before anti-HIV was first detected. From our data, however, it may not be concluded that HIV-ag is regularly detectable so early, because no samples were available in between. Anti-HIV development appears to overlap with HIV-ag indicating that no window phase, with both markers absent, exists. On the other hand, the majority of anti-HIV seroconverters did not show transient HIV-ag. A recent study (Gaines *et al.* 1987) on individuals with symptoms of primary HIV infection suggests that this may be caused by the long sampling interval in our studies since in that report transient HIV-ag was found in all cases. A significant proportion of acutely infected homosexual men became HIV-ag seropositive before anti-HIV seroconversion and remained so thereafter. Half of these men developed serious clinical disease confirming the previously described relationship between persistent HIV antigenaemia and the transition to AIDS. This high percentage suggests that persistence of HIV-ag in early infection has a worse short-term outcome than described for people developing HIV antigenaemia during later stages of infection. In homosexual men worsening disease states were associated with increasing percentages of HIV-ag seropositivity, resulting in a value of 86% among patients diagnosed as having AIDS. Both AIDS cases who could be studied longitudinally and who were persistently HIV-ag seronegative had HIV-ag in circulating immune complexes (Lange *et al.* 1987). These results indicated that HIV-ag can only be detected in free form by the present technique. In both Dutch haemophiliacs and i.v. drug users no or very few AIDS cases have been diagnosed paralleling the very low frequencies of HIV antigenaemia found. Finally it has been recently shown that AZT resulted in significant decreases in HIV antigen levels and concomitant clinical improvement (Chaisson *et al.* 1986). No data are available yet on the difference in infectivity of HIV antigen positive versus HIV antigen negative serum or plasma, although preliminary data indicate that cell-free virus is more frequently present in HIV-ag positive serum (Gaines

et al. 1987; Falk *et al.* 1987). The low frequency of HIV-ag in serum of Central African AIDS patients may result from the fact that the assay is unable to detect expression of viruses with a somewhat divergent gag gene (Ferns, Tedder & Weiss, 1987). Alternatively, 'Slim Disease' patients may express HIV antigen at the same rate as European or USA AIDS patients but excess of core specific immunoglobulins may trap core antigens in immune complexes as has been shown for HIV-ag negative Western AIDS cases.

Nine of the ten African patients tested had high levels of antibodies reactive with the HTLV-III gag gene product p24; an antibody generally absent from sera of European and American AIDS patients. In children a completely different picture emerged. Antibodies to HIV may be absent in HIV-infected children, however, HIV-ag is common during all stages of infection (Lange *et al.* 1986a; Borkowski *et al.* 1987; Tovo *et al.* 1987). This accords with the fact that significantly higher proportions of children get infected when exposed to the virus and a higher frequency of serious disease is seen within a short time of follow-up compared to adults. HIV-ag in the serum of a child proves that the virus circulates but has little predictive value for disease progression. HIV-ag in cerebro-spinal fluid has been shown previously to be of more use in this respect (Epstein *et al.* 1987).

In conclusion HIV-ag appears to be a useful marker in detecting early infection. Persistence of HIV-ag in serum among both children and adults bears a grave clinical prognosis and serves as a good selection criterion for antiviral therapy, at least in Europe and the USA. It remains to be determined why HIV-ag levels are so low among African AIDS cases. The preliminary data on significant decreases of HIV-ag levels after treatment with antiviral drugs (Chaisson *et al.* 1986) indicate that the HIV-ag EIA is a suitable tool (perhaps even more than viral culture from cells and comparable to viral culture from plasma) for evaluating the efficiency of antiviral drug treatment.

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