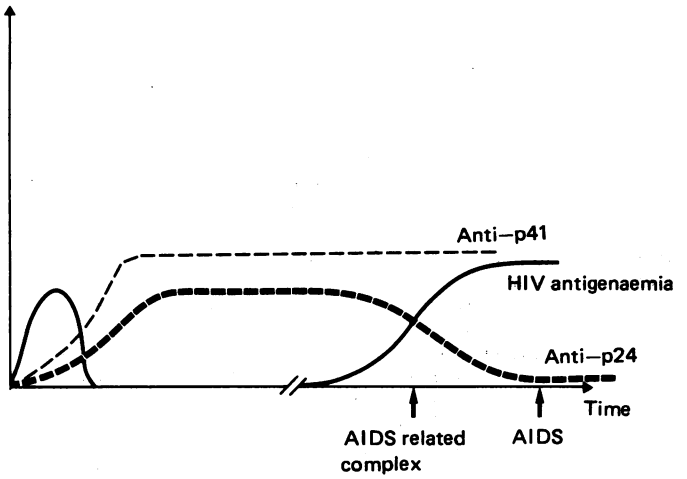

FIG 2—Serological profile of HIV infection.

patients, however, HIV antigen remains detectable after the primary infection[1] (Pedersen *et al*, unpublished).

The serological events during acute infection resemble those of other viral infections associated with antigenaemia, but the recurrence of antigenaemia and loss of anti-p24 during late stages of the infection are less readily explained. Loss of anti-p24 due to defective B cell function is unlikely to be the initial event, as the production of antibodies directed against other HIV antigens remains intact. More probably loss of anti-p24 is secondary to the formation of antigen-antibody complexes due to increasing amounts of antigen in the late stages of the infection. This hypothesis is supported by the finding of HIV antigen in polyethylene glycol precipitated immune complexes both in patients with anti-p24 and in patients without detectable anti-p24 in serum.[16]

Our study shows that some patients develop antigenaemia within two years of infection while others remain healthy without antigenaemia for up to seven years. The reasons for the different courses remain obscure. Further studies of the serological profile in HIV infection, with special attention paid to events preceding antigenaemia and loss of anti-p24, may establish the role of possible cofactors for the progression of clinical disease.

Recurrence of HIV antigenaemia seems to indicate a poor prognosis. Kaplan-Meier curves (data not shown) show that 49% of patients will develop AIDS within two years from the reappearance of antigenaemia. This must be added to the long list of clinical, histological, immunological, and serological indicators of a bad prognosis.[17] The ideal prognostic test must be simple to do and interpret. The antigen test fulfils these criteria and has the further advantage that it can be used in the form of a filter paper test,[8] which may be of value in developing countries.

This study was supported by grants from the Michaelsen Foundation and the Danish State Medical Research Council.

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(Accepted 21 July 1987)

Risk of AIDS related complex and AIDS in homosexual men with persistent HIV antigenaemia

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Abstract

One hundred and ninety eight men seropositive for human immunodeficiency virus (HIV) antibody and 58 HIV antibody seroconverters were studied for an average of 19·3 (SEM 0·5) months to assess the relation between HIV antigenaemia and the risk of developing the acquired immune deficiency syndrome (AIDS) and AIDS related complex. Forty (20·2%) of the 198 HIV antibody seropositive men were antigen positive at entry and remained so during follow up. Eight (13·8%) of the 58 HIV antibody seroconverters and 20 (12·7%) of the remaining 158 HIV antibody seropositive men became antigen positive during follow up, resulting in an end point attack rate for HIV antigenaemia of 14·3%. AIDS related complex was diagnosed in 25 (15·8%) of the HIV antigen negative men and in 14 (20·7%) of the HIV antigen positive men. AIDS was diagnosed in 15 men, resulting in an end point attack rate for AIDS of 23·9% in the HIV antigen positive group and 1·3% in the antigen negative group.

HIV antibody seropositive men without symptoms but with persistent HIV antigenaemia are at increased risk of developing AIDS and AIDS related complex.

Introduction

Human immunodeficiency virus (HIV) is the causative agent of the acquired immune deficiency syndrome (AIDS) and related

disorders.[1-4] Primary HIV infection is associated with transient changes in T4/T8 lymphocyte subsets, mainly due to an increase in the number of $T8^+$ lymphocytes.[5-7] Long term seropositivity for HIV antibodies is associated with inversion of the T4 to T8 ratio due to decreased numbers of $T4^+$ cells, in some cases accompanied by an increase in $T8^+$ cells.[8-11] Clinical disease develops in a large proportion of patients with long term seropositivity for HIV antibody.[10-13] In both transectional and longitudinal studies a grave clinical outcome appeared to be associated with the presence of HIV antigen and the decline of HIV core antibodies in serum.[14-17]

We report a prospective study carried out over two years in which we determined the prevalence and incidence of HIV antigenaemia among HIV antibody seropositive (n=198) and HIV antibody seroconverted (n=58) homosexual men. The onset of clinical disease, classified according to the system used by the Centers for Disease Control,[4] was studied in relation to the presence of HIV antigen in peripheral blood.

Subjects and methods

Between October 1984 and March 1986, 961 asymptomatic men living in and around Amsterdam and with at least two homosexual contacts in the preceding six months were enrolled in a prospective study of the prevalence and incidence of HIV infection and risk factors for AIDS. Epidemiological and clinical data were collected and blood sampled every three months. Of the 961 men, 723 were found to be HIV antibody seronegative and 238 HIV antibody seropositive in the first serum sample taken. The mean ages of the two groups were 34·9 (SEM 7·7) and 35·2 (6·9) years, respectively. During follow up 59 initially HIV antibody seronegative men seroconverted (end point attack rate for HIV antibody seroconversion 12·9% after 660 days). Forty HIV antibody seropositive men and one HIV antibody seroconverter failed to return for follow up at three or six months after entering the study. Sequential serum samples from the remaining 198 HIV antibody seropositive men and 58 HIV antibody seroconverters were therefore collected between October 1984 and October 1986 and tested for HIV antigen. The mean age of these two groups was 35·1 (SEM 6·5) years and mean duration of follow up 19·3 (SEM 0·5) months.

HIV antigen seropositivity was defined as the presence of HIV antigen detected in three or more sequential serum samples taken over at least six months. Eleven of the 58 HIV antibody seroconverters had HIV antigen transiently in serum before seroconversion and were included in the antigen negative group.

All subjects in the study were classified according to the Centers for Disease Control system for HIV infections.[4]

DETECTION OF HIV ANTIBODIES AND HIV ANTIGEN

Two commercially available enzyme linked immunosorbent assays (ELISA) with purified human T cell lymphotropic virus type IIIB used as antigen (Abbott Laboratories, Chicago; Vironostika Teknika, Organon, Oss, The Netherlands) were used to detect HIV antibody. Seropositivity was confirmed by immunoblotting, as described.[16]

Serum samples were assayed for HIV antigen in a solid phase immunoassay (Abbott Laboratories). Samples (200 µl) were incubated overnight at room temperature with human anti-HIV coated beads. Beads were then washed with distilled water, rabbit anti-HIV IgG was added, and the beads were incubated for four hours at 45°C. Following this beads were washed as before then incubated for a further two hours at 45°C with goat antirabbit IgG conjugated to horseradish peroxidase. After a final wash beads were transferred to tubes and *o*-phenylene diamine added. After 30 minutes at room temperature in the dark 1N sulphuric acid was added to each tube. Optical density at 492 nm was read by the Quantum Dual Wavelength Spectrophotometer (Abbott Laboratories). A sample was considered positive if its optical density was $\geq 0{\cdot}050$ plus the mean of five duplicate readings in normal human plasma.

The solid phase immunoassay is especially sensitive for the core antigen of HIV, detecting antigen in culture supernatant of 10^3 cells/l 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 radioimmunoprecipitation procedures detects p55/24 (core) strongly and gp120/41 (env) only faintly. Purified recombinant core antigen is detectable at 50 ng/l when spiked into serum or plasma, whereas purified recombinant env antigen is detectable at roughly 500 µg/l. We quantified HIV antigen by comparing the optical densities of the samples with optical densities of known quantities of purified HIV lysate. With use of this antigen as a reference the detection limit of the assay was approximately 20-30 ng/l.

STATISTICS

Life table attack rates were calculated by the actuarial method.[18] HIV antibody seroconverters were entered into intervals from the time of the first HIV antibody seropositive serum sample and HIV antigen seroconverters entered from the time of the first HIV antigen positive serum sample. Life table attack rates were compared by using the statistics of Lee and Desu.[19] Other statistical methods used were *t* tests and the χ^2 test.

Results

Table I and figure 1 give the detailed and summarised data on HIV antigenaemia and the development of clinical disease.

HIV ANTIGENAEMIA IN HIV ANTIBODY SEROPOSITIVE MEN

Eight (13·8%) of the 58 HIV antibody seroconverters became HIV antigen positive at or shortly after seroconversion (fig 1, table I; group 2). Forty (20·2%) of the 198 HIV antibody seropositive men were HIV antigen positive (group 5) at entry to the study and 20 (10·1%) became HIV antigen positive (group 4) during follow up. In all HIV antigen seropositive groups the serum HIV antigen concentration increased significantly over time (group 2 $p<0{\cdot}05$; group 4 $p<0{\cdot}002$; group 5 $p<0{\cdot}0001$) (table I). The end point attack rate after 660 days for HIV antigenaemia among HIV antibody seropositive men and HIV antibody seroconverters combined was 14·3% (fig 2).

CLINICAL DISEASE AND HIV ANTIGENAEMIA

AIDS related complex (Centers for Disease Control (CDC) classification IV-A) developed in 10 (20·0%) of the 50 seroconverters without persisting HIV antigenaemia (fig 1, table I; group 1) and in 2 (25·0%) of the eight seroconverters with persisting antigenaemia (group 2). Among the HIV antibody seropositive men 15 (10·9%) in the HIV antigen seronegative group (group 3), 5 (25·0%) of the HIV antigen seroconverters (group 4), and 7 (17·5%) of the HIV antigen seropositive men (group 5) developed AIDS related complex during follow up. Hence overall AIDS related complex developed significantly more frequently among HIV antigen seropositive men (groups 2, 4, and 5) than among HIV antigen seronegative men (groups 1 and 3) ($p<0{\cdot}00001$).

AIDS developed in 2 (25·0%) of the eight seroconverters with HIV antigenaemia (group 2), in 2 (1·4%) of the 138 seropositive men without HIV antigenaemia (group 3), in 2 (10·0%) of the 20 seropositive men who became HIV antigen positive (group 4), and in 9 (22·5%) of the 40 seropositive men with HIV antigenaemia at entry (group 5). Hence among the total group of 256 seropositive men 15 developed AIDS, representing an AIDS attack rate of 7·8% after 660 days (fig 3). Two cases of AIDS occurred among HIV

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TABLE I—*HIV antigenaemia and clinical disease in 256 homosexual men infected with HIV. (Study period October 1984 to October 1986)*

	HIV antibody seroconverters (n=58)		HIV antibody seropositive men (n=198)			
	Group 1 (antibody seronegative → antibody seropositive/ antigen seronegative)	Group 2 (antibody seronegative → antibody seropositive/ antigen seronegative → antigen seropositive)	Group 3 (antibody seropositive/ antigen seronegative)	Group 4 (antibody seropositive/ antigen seronegative → antigen seropositive)	Group 5 (antibody seropositive/ antigen seropositive)	Total
No (%) of men	50/58 (86·2)	8/58 (13·8)	138/198 (69·7)	20/198 (10·1)	40/198 (20·2)	256 (100·0)
Mean (SEM) duration (weeks) of:						
Antibody seropositivity	36·7 (5·2)	45·3 (16·0)	78·6 (6·4)	85·1 (19·1)	64·4 (2·9)	
Antigen seropositivity	—	35·3 (7·6)	—	38·3 (4·6)	64·4 (2·9)	
Mean (SEM) HIV antigen concentration (ng/l) in serum in:						
First antigen seropositive sample	—	159·1 (85·3)	—	58·6 (25·0)	232·5 (44·4)	172·6 (30·1)
Last antigen seropositive sample	—	547·6 (117·7)	—	160·0 (29·7)	410·5 (53·6)	354·1 (38·8)
No (%) of men with infections classified as:						
CDC I	23 (46·0)	5 (62·5)	—	—	—	28 (10·9)
CDC II*	16 (32·0)	1 (12·5)	78 (56·5)	8 (40·0)	11 (27·5)	114 (44·5)
CDC III*	24 (48·0)	3 (37·5)	43 (31·2)	5 (25·0)	13 (32·5)	88 (34·4)
CDC IV-A* (AIDS related complex)	10 (20·0)	2 (25·0)	15 (10·9)	5 (25·0)	7 (17·5)	39 (15·2)
CDC IV-C-1 (AIDS)	0	1 (12·5)	1 (0·7)	2 (10·0)	6 (15·0)	15 (5·9)
CDC IV-D (AIDS)	0	1 (12·5)	1 (0·7)	0	3 (7·5)	

CDC=Centers for Disease Control. *Classification at last visit.

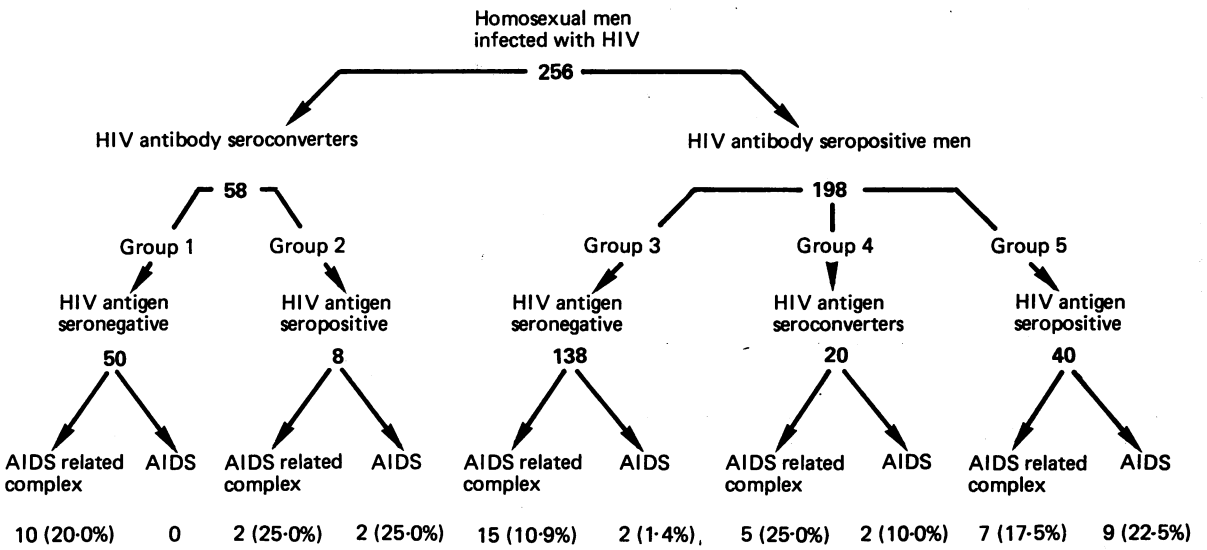

FIG 1—Diagrammatic summary of risk of AIDS related complex and AIDS in asymptomatic subjects with HIV antigenaemia.

antigen negative men (group 3) and 13 cases among HIV antigen positive men (groups 2, 4, and 5; $p<0{\cdot}00001$). The end point attack rate for AIDS after 660 days was 23·9% among HIV antigen seropositive men and 1·3% among HIV antigen seronegative men ($p<0{\cdot}00001$; D=23·292; fig 4).

The presenting diagnosis of AIDS was *Pneumocystis carinii* pneumonia (CDC IV-C-1) in 10 men, Kaposi's sarcoma (CDC IV-D) in four, and Burkitt's lymphoma (CDC IV-D) in one (table II). Nine of the 13 HIV antigen seropositive men with AIDS remained HIV antigen seropositive throughout. The mean duration of HIV antigenaemia before AIDS was diagnosed in this group was 59·8 weeks (range 9-82 weeks; table II). Two men with AIDS (cases 3 and 6) from the HIV antibody seropositive cohort became HIV antigenaemic 35 and 13 weeks after entering the study and five and 29 weeks before AIDS was diagnosed. The remaining two cases of AIDS (cases 5 and 9) were in men who initially were HIV antibody seronegative. They became HIV antigenaemic 12 and six weeks after HIV antibody seroconversion and 12 and 60 weeks before AIDS was diagnosed. In cases 1 and 2 (table II) HIV antigen remained undetectable throughout the study.

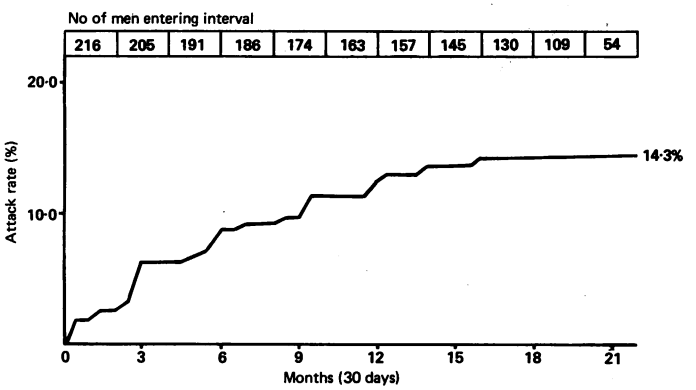

FIG 2—HIV antigen life table attack rate among HIV antibody seropositive homosexual men. (Study period October 1984 to October 1986.)

Discussion

A high proportion of subjects infected with HIV develop immunological and clinical abnormalities during long term follow up.[8-13] Previous studies have indicated that low values of HIV core antibodies in serum are associated with AIDS.[16 17] Declining levels of antibodies to core proteins may precede the development of AIDS by months to over a year.[16 17] Subsequent studies have shown that persistent HIV antigenaemia in conjunction with declining core antibodies may be associated with progression of disease.[14 15 21 22] This study extends our observations to the incidence of persistent HIV antigenaemia and the incidence of AIDS related complex and AIDS in relation to antigenaemia.

We found an increase in HIV antigen positivity among HIV antibody seropositive men with time resulting in an end point attack rate for HIV antigenaemia of 14·3% after about two years. The interval between HIV antibody seroconversion and HIV antigen seroconversion varied widely. Repeated quantitative tests for HIV core antibody may help in identifying people prone to develop detectable amounts of HIV antigen as a decline of HIV core antibodies sometimes precedes HIV antigenaemia (J M A Lange *et al*, submitted for publication). Declining amounts of HIV core

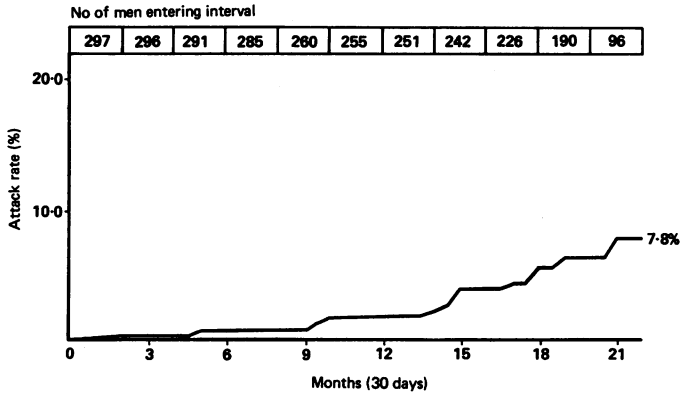

FIG 3—AIDS life table attack rate among HIV antibody seropositive homosexual men. (Study period October 1984 to October 1986.)

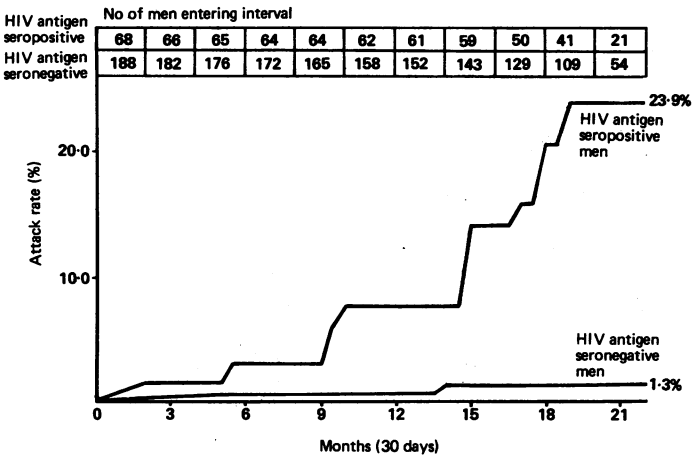

FIG 4—AIDS life table attack rates among HIV antibody/HIV antigen seropositive and HIV antibody seropositive/HIV antigen seronegative homosexual men. (Study period October 1984 to October 1986.)

TABLE II—*HIV antigenaemia and clinical disease in 15 cases of AIDS*

Case No	HIV antigen concentration (ng/l) First serum sample	HIV antigen concentration (ng/l) Last serum sample	Duration of HIV antigenaemia before diagnosis of AIDS (weeks)	Presenting diagnosis
1	0	0	—	Burkitt's lymphoma
2	0	0	—	*P carinii* pneumonia
3	0	26	5	*P carinii* pneumonia
4	583	47	9	Kaposi's sarcoma
5	0	350	12*	*P carinii* pneumonia
6	0	259	29	*P carinii* pneumonia
7	166	116	42	*P carinii* pneumonia
8	28	35	44	Kaposi's sarcoma
9	0	1071	60*	Kaposi's sarcoma
10	57	336	65	*P carinii* pneumonia
11	210	294	65	*P carinii* pneumonia
12	294	1176	75	*P carinii* pneumonia
13	40	151	77	*P carinii* pneumonia
14	275	280	79	*P carinii* pneumonia
15	231	589	82	Kaposi's sarcoma

*HIV antibody/HIV antigen seroconverter.

antibodies indicate trapping of these antibodies by HIV antigen in immune complexes.[23] The assay that we used detects "free" antigen remaining once the amount of HIV core antibody has been exhausted.

Most HIV antibody seropositive men without HIV antigenaemia remained symptom free during follow up. In addition, persistent generalised lymphadenopathy (CDC III) was as prevalent among HIV antigen negative as among HIV antigen positive men. AIDS related complex and AIDS, however, were seen more frequently among HIV antigen positive than among HIV antigen negative men. The incidence of AIDS in our study was some 20 times higher among men who were HIV antigen positive than among those who were HIV antigen negative.

Two cases of AIDS occurred in the HIV antigen seronegative group. One patient had Burkitt's lymphoma and subsequently died, and the other had *P carinii* pneumonia and remained well after treatment. In sequential serum samples from both subjects circulating immune complexes containing HIV antigen were detected.[23]

The duration of HIV antigenaemia before the diagnosis of AIDS varied from five to 82 weeks, ruling out any possibility of predicting the time interval between HIV antigen positivity and the development of AIDS. The same was true for the concentration of HIV antigen; high as well as low concentrations of HIV antigen were detected in those who went on to develop AIDS and in those who did not over the same period.

In conclusion our data show that the incidence of HIV antigen positivity increases with time and that the attack rate of AIDS is significantly higher among HIV antigen seropositive men than among HIV antigen seronegative men. The identification of these subgroups of HIV antibody seropositive and symptomless men with distinguishable risks for AIDS is important; it opens the way to predict future cases of AIDS more accurately and to select subjects infected with HIV for trials of antiviral drugs that effectively reduce the viral antigen load aiming at preventing AIDS rather than treating AIDS.

We thank Nel Albrecht-van Lent, Roos Lopez-Dias, Kiki Nittel, Emilie Nord, Margreet Bakker, Lia Smit, Sylvia Teengs, Marijke Roos, and David Mack for technical help and Riet de Krieger-Reumkens for preparing the manuscript. This study was partly supported by grant No 281026 of the Netherlands Foundation for Preventive Medicine.

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(Accepted 7 July 1987)