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EXPRESSION OF HUMAN IMMUNODEFICIENCY VIRUS ANTIGEN (HIV-Ag) IN SERUM AND CEREBROSPINAL FLUID DURING ACUTE AND CHRONIC INFECTION

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Summary Human immunodeficiency virus antigen (HIV-Ag) was detected in the serum of most adult (13/16) and paediatric (6/6) AIDS patients and rarely in the serum of symptomless seropositive controls (1/13). It was present in the cerebrospinal fluid (CSF) of all 5 children and most (5/9) adults with AIDS-related encephalopathy, but not in the CSF of 13 symptomless seropositive controls, of whom 8 had antibody in the CSF. A longitudinal study of 1 of the controls with antibody in the CSF showed that HIV-Ag in CSF was present transiently before the occurrence of antibody in the CSF. In serial samples of serum from 35 men who seroconverted HIV-Ag was detected in 11 persons—in 5 before seroconversion and in 6 after. 3 of the 6 who became antigenaemic after seroconversion remained so for the rest of the follow-up. AIDS was diagnosed in 1 patient, 3 months after HIV-Ag was first detected in serum and 6 months after seroconversion. The findings suggest that HIV-Ag appears early and transiently in primary HIV infection. Antibody production follows, after which HIV-Ag may disappear. Its persistence or reappearance seems to correlate with clinical, immunological, and neurological deterioration.

Introduction

HUMAN immunodeficiency virus (HIV), the agent that causes the acquired immunodeficiency syndrome (AIDS),[1-3] is neurotropic and lymphotropic.[4-7] It can be readily isolated from phytohaemagglutinin-stimulated peripheral blood mononuclear leucocytes of individuals with AIDS or at risk of AIDS.[8,9] It can also be isolated from the cerebrospinal fluid (CSF) and neural tissues of patients with neurological symptoms related to AIDS.[6,7,9]

Primary HIV infection is often associated with atypical febrile illness[10-12] and in some cases with acute encephalopathy[13] and meningitis.[6] Chronic HIV infection may result in AIDS and AIDS-related progressive encephalopathy in both children and adults.[6,7,10,14]

Serum antibodies to HIV (anti-HIV) appear several weeks to months after primary infection.[11,12] Central nervous system synthesis of HIV-specific antibody has been reported in persons with AIDS-related neurological symptoms as well as in symptomless seropositive individuals.[15,16] We have used a new, sensitive HIV antigen (HIV-Ag) test[17] to assay serum/CSF pairs from symptomless seropositive individuals and paediatric and adult AIDS patients with and without neurological symptoms. We also report on findings in serial serum samples from 6 homosexual men who seroconverted while under surveillance. In one patient serial CSF specimens were available for tests.

Subjects and Methods

Subjects

Serum/CSF pairs were collected from:

1. 16 adult homosexual patients with AIDS presenting with opportunistic infections, Kaposi's sarcoma, or both, who were admitted to the Academic Medical Center, University of Amsterdam. Their neurological symptoms were assessed by one of us (H. S.).

2. 13 adult HIV-seropositive homosexual males without AIDS or AIDS-related complex (ARC) who were participating in a diagnostic and therapeutic neurosyphilis programme. The absence of neurological symptoms was confirmed by one of us (E. Ch. W.). In 1 of these patients serial serum/CSF pairs were available for tests.

3. 6 children with AIDS, identified according to the Centers for Disease Control criteria. All were admitted to the department of paediatrics of the University of Medicine and Dentistry of New Jersey and examined neurologically by one of us (L. G. E.). All except 2 (patients 18 and 20) were born to intravenous drug-using parents and to seropositive mothers. Patients 18 and 20 were infected by blood transfusion.

35 individuals who were taking part in a prospective study on the prevalence and incidence of HIV infection and risk factors for AIDS seroconverted to HIV while under surveillance. Between October, 1984, and April, 1985, 741 homosexual and bisexual men with multiple male sexual partners and living in and around Amsterdam were enrolled and seen every 3 months. Epidemiological and clinical data were collected and blood was

taken for immunology (T-cell subsets) and serology. Of the 741 men, 508 (69%) were found to be HIV-antibody negative. During follow-up, up to the end of February, 1986, 40 seroconverted. This report is of the first 35.

HIV Antibody (HIV-Ab) and Antigen (HIV-Ag)

An enzyme-linked-immunosorbent assay (ELISA)[10] with purified HTLV-IIIB as antigen (Vironostika, Organon, Oss, The Netherlands) was used to detect HIV-Ab.[10] Antibody titres were determined by testing two-fold dilutions of serum and CSF and the titre was taken as that dilution for which the 2 logarithm corresponded to an optical density at 450 nm of (OD maximum + OD minimum)/2. The specificity and sensitivity for HIV was confirmed by immunoblotting,[18] and reactivity with the major core protein p24 or the transmembrane protein gp41, with or without other viral proteins, was taken as a positive result. The serum and CSF of AIDS patients reacted predominantly with gp41; the serum and CSF of all others reacted predominantly with p24.

Samples were assayed for HIV antigen in a solid-phase sandwich-type enzyme immunoassay, with polyclonal antibodies to HTLV-III being used as capture and probe antibodies and a labelled second-antibody being used to identify a positive reaction. Briefly, the assay was done as follows: 200 µl of sample was incubated overnight at room temperature with polystyrene beads coated with human anti-HTLV-III-IgG. Beads were washed with distilled water, and rabbit anti-HTLV-III-IgG was added to each, then incubated for 4 h at 45°C. Beads were washed as before, then incubated for 2 h at 45°C with goat anti- rabbit IgG conjugated to horseradish peroxidase. After a final wash, beads were transferred to tubes and *o*-phenylene diamine was added to each. After 30 min at room temperature in the dark, 1 ml of 1 N H_2SO_4 was added to each tube to stop the enzyme reaction. The absorbance was read at A_{492} with the quantum dual wavelength spectrophotometer. A result was judged to be positive if the OD was greater than 0·050 plus the mean of 5 replicates of normal human plasma.

The specificity of the assay was established by testing tissue culture supernatants from cell-lines infected with various viruses, including Epstein-Barr virus, two herpes strains, gibbon ape leukaemia virus, and HTLV-I. No false-positive reactions were seen with these cell-lines at 10^7 cells/ml, whereas antigen in the culture supernatant of HTLV-III-infected HT-9 cells was detectable at a concentration of 10^3 cells/ml. Uninfected HT-9 cell supernatants were also negative. 200 serum and plasma samples from donors not at risk of AIDS showed no false-positive reactions. The assay is most sensitive for the major core protein p24 of the virus and can detect approximately 50 pg/ml of this purified protein.

HIV antigen was quantified by comparing the ODs of the serum samples with ODs of known quantities of purified HTLV-III lysate. Since the assay is linear to 2·0 OD, the sensitivity of the assay in this instance was approximately 0·1 ng/ml, with purified HTLV-III lysate being used as reference.

Results

19 (86%) of 22 adult and paediatric patients with AIDS had detectable amounts of HIV-Ag in the serum and 12 (55%) of these had HIV-Ag in the CSF (table I). In contrast, 1 (8%) of 13 seropositive symptomless individuals had HIV-Ag in the serum and none of these 13 had HIV-Ag in the CSF. All 22 AIDS patients with or without neurological symptoms had anti-HIV in the serum and 18 of the 22 had anti- HIV in the CSF (table II).

The quantity of HIV-Ag ranged from 0·5 to 27·5 ng/ml in serum, and from 0·1 to 7·6 ng/ml in CSF. HIV-Ag was detectable in the CSF of 5 (56%) of 9 adults and of all 5 children with progressive encephalopathy. 1 adult (patient 7) with progressive encephalopathy had HIV-Ag in the CSF but not in serum, and another (patient 16) had more HIV-Ag in CSF than in homologous serum. 2 adults without progressive encephalopathy had HIV-Ag in the CSF. In 3 children with progressive encephalopathy (patients 19, 21, and 22), the quantity of HIV-Ag exceeded the quantity in the homologous serum.

TABLE I—HIV ANTIGEN AND ANTIBODY IN SERUM AND CEREBROSPINAL FLUID OF PATIENTS WITH AIDS AND AT RISK OF AIDS

Category	No (%) with HIV-Ab in:		No (%) with HIV-Ag in:	
	Serum	CSF	Serum	CSF
AIDS (n=22)	22 (*100*)	18 (*82*)	19 (*86*)	12 (*55*)
Symptomless (n=13)	13 (*100*)	8 (*62*)	1 (*8*)	0 (*0*)

TABLE II—HIV ANTIGEN AND ANTIBODY IN SERUM AND CEREBROSPINAL FLUID OF PAEDIATRIC AND ADULT CASES OF AIDS

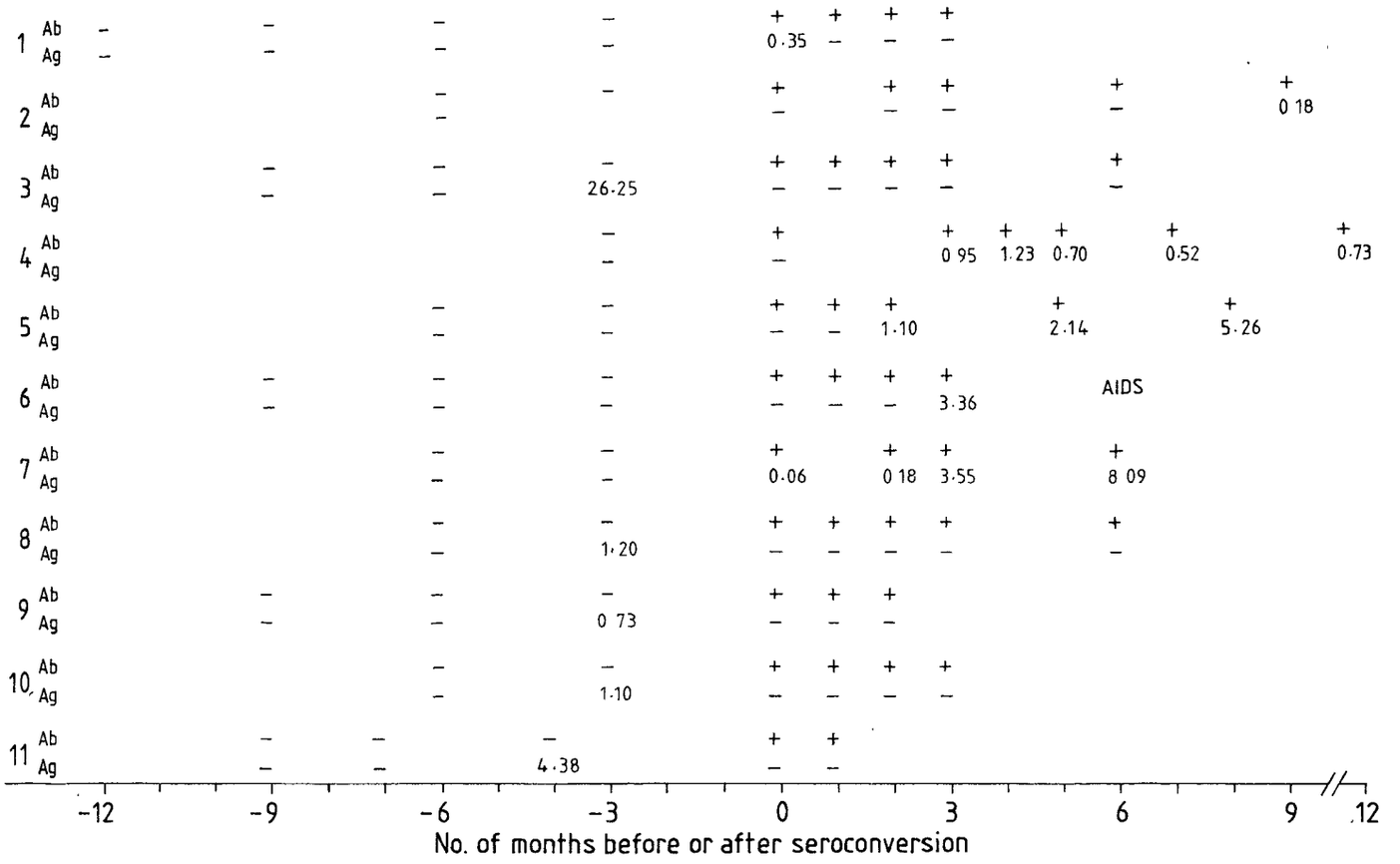
Patient no (age in yr/sex)	HIV antibody in:		HIV antigen (ng/ml) in:		PE/OI
	Serum	CSF	Serum	CSF	
1 (46/M)	+	+	27·5	–	+/+
2 (42/M)	+	+	4·7	0·2	+/–
3 (33/M)	+	–	0·5	–	–/+
4 (46/M)	+	+	4·6	1·1	–/+
5 (38/M)	+	+	0·5	–	+/+
6 (54/M)	+	+	2·7	–	–/+
7 (36/M)	+	+	–	1·7	+/–
8 (35/M)	+	+	–	–	+/–
9 (41/M)	+	+	5·1	0·3	–/–
10 (39/M)	+	+	–	–	–/+
11 (25/M)	+	–	4·7	3·4	+/–
12 (40/M)	+	+	7·4	–	–/–
13 (38/M)	+	+	0·8	–	–/–
14 (32/M)	+	+	3·3	0·7	+/–
15 (25/M)	+	+	4·6	–	+/–
16 (38/M)	+	+	2·7	4·7	+/–
17 (3/F)	+	+	9·0	–	–/–
18 (1·9/M)	+	–	17·3	3·9	+/–
19 (2·5/F)	+	–	3·6	7·6	+/–
20 (2·6/F)	+	+	3·9	0·1	+/–
21 (6/M)	+	+	4·2	5·2	+/–
22 (2/M)	+	+	0·7	1·4	+/–

*PE = progressive encephalopathy; OI = opportunistic infection of the central nervous system; + = present; – = absent.

HIV-Ag was detected in the sera of 11 (31%) of the 35 men who became anti-HIV-positive while under surveillance (see accompanying figure). 5 subjects (patients 3, 8, 9, 10, and 11) were HIV-Ag positive 13–16 weeks before antibodies appeared, and HIV-Ag levels ranged from 0·73 to 26·25 ng/ml.

6 subjects (patients 1, 2, 4, 5, 6, and 7) were seropositive when HIV-Ag was first detected. 2 of these (patients 1,7) became antigen and antibody positive almost simultaneously. In 1 of these (patient 7) HIV-Ag was detected in all follow-up sera after seroconversion, with HIV-Ag levels rising from 0·06 to 8·09 ng/ml. In patient 1, HIV-Ag was detected on only one occasion (0·35 ng/ml).

HIV-Ag appeared for the first time in sera taken 6 weeks, 3 months, 3 months, and 9 months after seroconversion in 4 subjects (patients 5, 4, 6, and 2, respectively). 1 of these (patient 5) remained antigenaemic until the end of the observation period, with antigen levels ranging from 1·10 to 5·26 ng/ml, while in another (patient 4) HIV-Ag levels remained constant at approximately 0·7 ng/ml. In another (patient 6) AIDS (with *Pneumocystis carinii* pneumonia) was diagnosed 3 months later. Shortly after seroconversion this man also showed serological evidence of recent syphilis and hepatitis B.



Order of appearance of HIV antigen and antibody in 11 patients.

Ab = anti-HIV; + = present; – = absent; Ag = HIV-Ag (ng/ml)

Sequential serum/CSF samples were available from 1 symptomless seropositive man (table III). This patient seroconverted in 1983 and antibodies in the CSF became detectable in 1984. HIV-Ag was detected only in the CSF sample obtained before the appearance of antibody in the CSF.

Discussion

HIV-Ag was detected in the serum of most adult and paediatric AIDS patients and only rarely in seropositive individuals without AIDS. Thus the presence of antigen in serum of chronically HIV infected individuals seems to be related to severe immunodeficiency and might be the cause of the decline in antibody titre to the major core protein (p24) that has been reported in patients with AIDS.[18-20]

HIV-Ag in CSF did not reflect leakage of viral protein or virus through the blood-brain barrier as illustrated by patients with extremely high levels of HIV-Ag in the serum and no HIV-Ag in CSF (table II, patients 1 and 17). The presence of larger quantities of HIV-Ag in the CSF than in the serum (table II, patients 7, 16, 19, 21, and 22) indicates that HIV is expressed in the central nervous system. HIV-Ag was present in the CSF of all children and most adults with AIDS-related progressive encephalopathy, whereas it was not detectable in the CSF of any seropositive symptomless controls. The relation between HIV-Ag in the CSF and progressive encephalopathy in children thus seems to be very strong. In adults, the pattern was less consistent—HIV-Ag was absent in the CSF of 4 of 9 patients with progressive encephalopathy and present in 2 of 7 patients without the encephalopathy. The occurrence of progressive encephalopathy in the absence of CSF HIV-Ag might be due to the effect of confounding factors such as opportunistic infections of the brain often seen in adults or to the sensitivity of the assay. Conversely, the detection of HIV-Ag in the CSF of patients without progressive encephalopathy may reflect a presymptomatic stage of HIV infection of the brain. Although Ho et al[6] showed that virus isolation from the CSF correlated strongly with adult AIDS-related progressive encephalopathy, virus isolation from neural tissue or CSF is subject to an amplification effect while direct demonstration of antigen is not. The quantity of HIV-Ag in the CSF is probably a more accurate reflection of the extent of viral expression in the brain.

HIV-Ag was detected in the serum of 11 patients who seroconverted while under surveillance. In 6 persons antigen was found only once, before or at the time of seroconversion, which suggests that persons may be infectious before seroconversion, as suggested previously.[12] The failure to detect antigen at the time of seroconversion and in later serum samples in these men may signify decreased viral replication. This may explain why no HIV-Ag was found in most symptomless seropositive individuals.

TABLE III—HIV ANTIGEN AND ANTIBODY IN SERUM AND CEREBROSPINAL FLUID IN A MAN WHO SEROCONVERTED

	HIV antibody titre in:		HIV antigen (ng/ml) in:	
Date	Serum	CSF	Serum	CSF
Nov 30, 1982	–	–	–	–
Aug 2, 1983	140	–	–	0·16
May 10, 1984	211	12	–	–
May 9, 1985	280	23	–	–

– = undetected.

After seroconversion 3 persons remained HIV antigenaemic for the rest of the follow-up period. Patient 6 became antigenaemic 3 months after seroconversion and was diagnosed as having AIDS 3 months later.

Whether AIDS-related complex or AIDS is more likely to develop in those persons who remain or become antigenaemic after seroconversion is being investigated.

The longitudinal study of a man who seroconverted showed that in acute infection HIV-Ag was present transiently in the CSF before the appearance of antibodies in the CSF. Antibodies produced within the CNS in symptomless individuals[16] might thus reflect past rather than current infection of the CNS.

The following tentative conclusions can be drawn from the data presented. Some and perhaps most HIV-infected individuals have viral antigen(s) circulating in serum before antibody production. Depending on viral expression, and time of testing, this antigen may be detectable. In early phases of the infection, the antigen may be detectable in the CSF with or without neurological symptoms. The disappearance of antigen from the serum of some individuals after seroconversion may reflect a latent phase of HIV infection. Some individuals remain antigenaemic, while in others the antigen reappears in the serum. The findings in patient 6 (table III) may be important since they could mean that the appearance of HIV-Ag in serum indicates a worsening of the illness—ie, progression to AIDS. The fact that the vast majority of AIDS patients in our series had HIV-Ag in the serum supports this notion. Similarly, persistence of HIV-Ag in the CSF most probably reflects severe CNS involvement. Epidemiological studies of high-risk groups may answer these questions.

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References continued at foot of next column

EFFECT OF RUBBERS AND THEIR CONSTITUENTS ON PROLIFERATION OF LEGIONELLA PNEUMOPHILA IN NATURALLY CONTAMINATED HOT WATER

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Summary The proliferation of *Legionella pneumophila* serogroups 1, 9, and 10 in naturally contaminated hot potable water was measured after the addition of various rubbers and their constituents. When compared with controls 9/14 rubbers and 15/30 constituents shortened lag time for growth of *L pneumophila* at 37°C and, produced a 10–100 000 fold increase in the number of *L pneumophila* organisms. In most experiments this increase was due mainly to the growth of *L pneumophila* serogroup 10, which predominated in all fresh water samples (47–53°C); in others a shift in predominance from serogroup 10 to serogroup 1 occurred. Rubbers containing thiuram did not enhance the growth of *L pneumophila*, while the addition of thiuram alone inhibited growth. Therefore thiuram-containing rubbers should be used in water systems.

Introduction

MANY nosocomial infections caused by *Legionella pneumophila* have been associated with water supplies contaminated with this organism.[1] Domestic water supplies can also be sources of *L pneumophila* infection.[2] Rubbers have been implicated as loci of growth of *L pneumophila* in water supplies.[3-4] We have shown that *L pneumophila* serogroups 1, 9, and 10 proliferate in the hot water system of our hospital and that the predominating serogroup changes with time.[1,5] Our preliminary laboratory experiments have shown that the growth patterns for these serogroups in water samples from the same hot water system changed when they were incubated in rubber-stoppered infusion bottles at 37°C. Since no nutrients were added to these samples we hypothesised that rubber constituents, which might leach into the water during intermittent shaking, provided the

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