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DURATION OF HUMAN IMMUNODEFICIENCY VIRUS INFECTION BEFORE DETECTION OF ANTIBODY

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Summary To estimate the duration and frequency of the period of HIV infection without detectable antibody, modelling techniques were applied to results of detection of HIV DNA by means of the polymerase-chain reaction (PCR) and to data from cases in published reports. PCR was carried out with *gag* and *env* region primers on samples from 27 homosexual and 12 haemophilic men for whom stored samples were available from before and after seroconversion; serum was also tested for p24 antigen by antigen-capture enzyme immunoassay. HIV DNA was detectable before seroconversion in 4 men; in all 4 PCR was positive only in the seronegative sample taken closest to the time of seroconversion. In 3 men antigen was detected before seroconversion; in each case HIV DNA was also detected. By a Markov model, the time from infection with HIV (as assessed by detection of HIV DNA) to first detection of HIV antibody was estimated to be 2.4 (SE 2.1) months for the median individual. Modelling of cases of HIV infection with known exposure in published reports gave a median estimate of 2.1 (0.1) months from exposure to antibody detection, and 95% of cases would be expected to seroconvert within 5.8 (0.6) months. HIV infection for longer than 6 months without detectable antibody seems uncommon.

Introduction

INFECTION with the human immunodeficiency virus (HIV) is usually identified by recognition of specific antibody to the virus, but in some people virus can be detected before the appearance of antibody by culture or detection of antigen.¹⁻⁶ HIV DNA can also be detected in peripheral blood mononuclear cells in the absence of HIV antibody.^{7,8} In most people with known HIV exposure who have been followed prospectively, detectable antibody has developed within 6 months,^{1,9} but Imagawa et al⁸ have reported cases of HIV infection for as long as 35 months without detectable antibody.

The length of time that a person is infected with HIV before antibody is detectable and the frequency of long antibody-negative periods have important public health and clinical implications. HIV-infected people who have sexual contact or donate blood during this period may infect others despite negative serological tests.¹⁰ Defining the time to seroconversion would also help health professionals to reassure seronegative people who have been exposed to HIV that they are not infected.

Infection with HIV results in incorporation of HIV DNA into host cells. Although HIV may be integrated into as few as 1 in 10⁴-10⁵ peripheral blood mononuclear cells,¹¹ HIV DNA in such a small percentage of cells can be detected by means of the polymerase chain reaction (PCR) technique.^{7,8,12,13} We have previously shown the greater sensitivity of PCR rather than viral culture for detection of HIV.¹⁴

We used PCR to analyse peripheral blood mononuclear cells from 39 men before and after seroconversion to HIV for evidence of HIV DNA. Since integration of HIV DNA into host genome in vitro occurs rapidly after infection,¹⁵ we assume that the PCR-positive, antibody-negative period is essentially the same as the period from infection to seroconversion. We used the results of PCR detection of HIV DNA to estimate the time from HIV infection to the development of detectable antibody to HIV. This estimate is supported by analysis of cases of HIV infection with a known date of exposure in published reports.

Methods

Cell and serum specimens were collected prospectively between 1984 and 1988 from participants in two studies of homosexual and bisexual men (the Boston Partners Study and the San Francisco City Clinic Cohort Study) and from men with haemophilia in the Hemophilia/AIDS Collaborative Cohort Study. Design and recruitment of participants for these studies have been described previously.¹⁶⁻¹⁸ All men in the studies who seroconverted between 1984 and 1988 were included in this study.

Serum specimens were tested with commercial enzyme immunoassays (Abbott Laboratories, Chicago, Illinois, or Genetic Systems, Seattle, Washington) and confirmed by immunofluorescence assay or western blot interpreted by standard criteria.¹⁹ Antigen-capture assay for HIV p24 antigen used a sandwich enzyme immunoassay with polystyrene beads coated with human anti-HIV (Abbott). PCR was carried out as previously described,¹⁴ with the following modifications: cells were lysed with a buffer containing 10 mmol/l "tris"-HCl, pH 8.3, 6 mmol/l magnesium chloride, 50 mmol/l potassium chloride, 0.45% 'NP 40', 0.45% 'Tween 20', 1 mg/ml gelatin, and 100 µg/ml proteinase K. The DNA preparation was incubated at 56°C for 60 min, then at 95°C for 10 min to inactivate proteinase K. A primer pair for the *gag* region of HIV-1, SK38/39,¹⁴ was used to subject 25 µl DNA preparation (corresponding to 150 000 cells) to 35 rounds of PCR in a volume of 100 µl. Amplified DNA was detected by means of the end-labelled *gag*-specific oligomer SK19.¹⁴ A second primer pair, SK68/69,¹⁴ for the *env* region of HIV-1, was used to confirm all samples with an end-labelled *env*-specific oligomer, SK70,¹⁴ to detect the amplified *env* region product.

Cases of HIV infection whose date of exposure could be defined to within 1 week were identified from published reports. All cases of HIV infection by any route of exposure were reviewed. There were many patients who were antibody positive when first tested but were first tested more than 6 months after exposure; they contributed little to the Markov estimates and were therefore excluded. We also excluded cases when we thought passive transfer of HIV antibody from blood donor to recipient had resulted in a positive enzyme immunoassay.

Since the progression from HIV infection to antibody detection is a staged process, the data were analysed by a Markov model.^{20,21} Such a model divides an ordered clinical process into periods defined by external criteria. Since the time of transition from one stage to the next, and therefore the exact length of each stage, is not usually known, statistical procedures are used to estimate the time when the transition occurred. In this analysis, stage 1 was defined as the period when the individual was negative by PCR and for antibody; stage 2 when the individual was positive by PCR and antibody negative; and stage 3 when the individual was positive by PCR and for antibody. Results are expressed as a point estimate (with SE) of the length of time that the median individual (and an individual on the 95th centile) would be expected to remain in a given stage; the stage of interest is the PCR-positive, antibody-negative stage. This approach is particularly useful when data are interval-censored (ie, the exact date of first appearance of HIV DNA and that of first detectable HIV antibody are not known; what is known is that the events occurred between defined sampling times).

Results

Of the 49 men from the two homosexual/bisexual studies and the haemophilic patient study who had documented seroconversion to HIV between 1984 and 1988, peripheral blood mononuclear cells from before seroconversion of 39 (80%) were available for study with the PCR technique. 35 of the 39 men (90%) were PCR negative 5-39 months before the first antibody-positive test (see table); 6 men also had samples available from 5-19 months before their last seronegative sample, and all were PCR negative. In 4 of the 39 (10%) HIV DNA was detected between 5 and 21 months before the first positive antibody test (table). In 2 of the 4

DETECTION OF HIV DNA IN PERIPHERAL BLOOD MONONUCLEAR CELLS BEFORE AND AFTER DETECTION OF HIV ANTIBODY

Patient	PCR at latest sample before seroconversion	Time (mo) to postseroconversion sample	Patient	PCR at latest sample before seroconversion	Time (mo) to postseroconversion sample
1	-	6	21	-	14
2	-	11	22	-	14
3	-	8	23	-	34
4	-	10	24	-	21
5	-	9	25	-	22
6	-	7	26	-	12
7	-	12	27	-	39
8	+	5	28	-	38
9	-	23	29	-	11
10	-	26	30	-	14
11	-	18	31	-	12
12	-	16	32	+	11
13	-	17	33	-	19
14	-	19	34	+	12
15	-	12	35	-	12
16	+	21	36	-	13
17	-	12	37	-	37
18	-	7	38	-	35
19	-	25	39	-	16
20	-	6			

Patients 1-27 were homosexual men; patients 28-39 were men with haemophilia.

men an additional sample that had been collected before the PCR positive sample (5 months and 13 months) was available; both samples were PCR negative. Of 23 patients who were tested after HIV antibody had developed, 19 were PCR positive, and 4 were PCR negative. Results with the *gag* and *env* primer pairs were concordant for all samples, positive and negative.

In 33 of the 36 (92%) patients tested no antigen was detected before seroconversion. Antigen was detected 5-21 months before antibody in 3 patients: all were PCR-positive at the same time.

Markov analysis of the time from detection of HIV DNA by PCR to the detection of HIV antibody estimated a median duration of 2.4 (SE 2.1) months. The time after which 95% of cases would be predicted to have seroconverted was 10.3 (9.3) months.

For comparison we reviewed reports of HIV-infected patients with a known exposure date. The estimates presented are derived from analysis of 45 cases: 7 due to transfusion of contaminated blood;^{2,22,23} 8 to organ transplantation from an infected donor;^{2,24-27} 17 to administration of contaminated factor VIII;^{1,28} 7 to needlestick injury;²⁹⁻³⁴ 3 to mucocutaneous exposure to contaminated blood;^{35,36} 2 to sexual contact with an HIV-infected partner;^{3,37} and 1 to sharing of contaminated equipment for intravenous drug abuse.³ The model estimated the time from HIV exposure to detection of HIV antibody to be 2.1 (0.1) months for the median individual and 5.8 (0.6) for an individual on the 95th centile.

Discussion

Episodes of HIV infection lasting 6 months or longer without the detection of antibody to HIV were first reported in 1987.⁴ Several groups of investigators have since concluded that such episodes are rare,^{9,38,39} but Imagawa and colleagues have suggested that the phenomenon may be more common.⁸ However, Imagawa and colleagues studied only subjects in whom seroconversion had not occurred despite extensive high-risk activity, which might have

caused them to overestimate the frequency and duration of the virus-positive, antibody-negative state. To avoid such sampling bias, we tested 80% of all seroconverters found in three separate studies over a 4-year period. In a separate study we also carried out PCR on cell samples from 89 seronegative men in the Boston and San Francisco studies who were at especially high risk of HIV infection and did not find any who were HIV-infected (unpublished). Therefore, our analysis of the virus-positive, antibody-negative state in seroconverters will not be an underestimate because of failure to include infected subjects who have not yet seroconverted.

PCR is more sensitive than virus culture in detecting HIV,¹⁴ and can detect 1 virus copy in 10^4 to 10^5 cells.^{7,12,13} Sensitivity of detection is increased by the use of several primer pairs and probes specific for different, highly conserved regions of the HIV genome.¹⁴ Despite the use of multiple primers, however, people positive for HIV antibody but without detectable HIV DNA by PCR have been reported^{12,14} and were seen in our study. Though it is possible that rare antibody-negative, DNA-positive samples might not be detected by PCR, such cases would not substantially alter the Markov estimates.

We estimated the duration of the PCR-positive, antibody-negative period by means of a staged Markov model, because the transitions to PCR positivity and to antibody positivity were known to have occurred only between the dates of the samples obtained (interval censoring). Since samples were obtained without regard to clinical status, we assumed that the likelihood of transition from one stage to the next was the same for all months during the interval.

We corroborated our estimate of the PCR-positive, antibody-negative period by analysing cases from published reports of HIV infection resulting from a known exposure. A potential bias exists in such a survey because cases of infection persisting for several years without the detection of antibody might not yet have been reported. We think this possibility is unlikely for two reasons. First, cases of chronic infection without the development of detectable antibody would be of substantial public health interest, and would probably be over-reported rather than under-reported; however, no cases of HIV infection with a defined exposure resulting in infection without the eventual development of antibody have been reported. Second, in a prospective study of health care workers exposed to HIV, no delayed recognition of infection was seen in 250 exposed persons who were followed up for at least 24 months.³⁴

The estimate of time from exposure to seroconversion of the published cases was similar to that estimated from the PCR results. It is, however, much more precise because in the case-reports only one of the two transition points was interval-censored (ie, the exact date of exposure was known). The time at which 95% of the reported cases could be expected to have seroconverted was 5.8 (0.6) months after exposure, compared with an estimated time of 10.3 (9.3) months by the PCR analysis. The large standard error of the estimate derived from the PCR results means that we cannot conclude that the interval was significantly different. This difficulty arises because only 4 samples were PCR positive before the detection of antibody. Until more experimental data are available, we believe that the estimate from the published cases should be used as a reasonable indication of the time by which 95% of HIV-infected persons can be expected to have seroconverted.

We conclude that detection of HIV DNA in peripheral blood mononuclear cells by PCR precedes the appearance of antibody and can be a useful adjunct to HIV diagnosis when antibody is not detected but clinical suspicion is high and early diagnosis is important. However, the assay requires sophisticated laboratory facilities and is not yet suitable for widespread use. Detection of antibody to HIV with enzyme immunoassay kits now available should remain the standard method of diagnosis of HIV infection. Analysis of available data suggests that in about 95% of all individuals exposed to HIV who become infected, antibodies detectable with currently available tests will develop within 6 months. Some subjects may need a longer period of observation to establish whether or not infection has occurred.

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REFERENCES

1. Simmonds P, Lainson FAL, Cuthbert R, Steel CM, Peutherer JF, Ludlam CA. HIV infection and antibody detection: variable responses to infection in the Edinburgh haemophilic cohort. *Br Med J* 1987; 296: 593-98.
2. Ward JW, Schable C, Dickinson GM, et al. Acute human immunodeficiency virus (HIV) infection: antigen detection and seroconversion in immunosuppressed patients. *Transplantation* 1989; 47: 722-24.
3. Ho DD, Sarngadharan MG, Resnik L, et al. Primary human T-lymphotropic virus type III infection. *Ann Intern Med* 1985; 103: 880-83.
4. Ranki A, Valle S-L, Krohn M, et al. Long latency precedes overt seroconversion in sexually transmitted human-immunodeficiency-virus infection. *Lancet* 1987; ii: 589-93.
5. Kessler HA, Blaauw B, Spear J, et al. Diagnosis of human immunodeficiency virus infection in seronegative homosexuals presenting with an acute viral syndrome. *JAMA* 1987; 258: 1196-99.
6. Goudsmit J, de Wolf F, Paul DA, et al. Expression of human immunodeficiency virus antigen (HIV-Ag) in serum and cerebrospinal fluid during acute and chronic infection. *Lancet* 1986; ii: 177-80.
7. Loché M, Mach B. Identification of HIV-infected seronegative individuals by a direct diagnostic test based on hybridisation to amplified viral DNA. *Lancet* 1988; ii: 418-21.
8. Imagawa DT, Lee MH, Wolinsky SM, et al. Human immunodeficiency virus type 1 infection in homosexual men who remain seronegative for prolonged periods. *N Engl J Med* 1989; 320: 1458-62.
9. Nekwei W, Colebunders RL, Bahwe Y, Lebughe I, Francis H, Ryder R. Acute manifestations of HIV infection following blood transfusion. In: IV International Conference on Acquired Immunodeficiency Syndrome. Abstracts, vol 2. Stockholm: Swedish Ministry of Health and Social Affairs, 1988: 350.
10. Ward JW, Holmberg SD, Allen JR, et al. Transmission of human immunodeficiency virus (HIV) by blood transfusions screened as negative for HIV antibody. *N Engl J Med* 1988; 318: 473-78.
11. Harper M, Marselle L, Gallo R, et al. Detection of lymphocytes expressing human T-lymphotropic virus type III in lymph nodes and peripheral blood from infected individuals by in situ hybridization. *Proc Natl Acad Sci USA* 1986; 83: 722-26.
12. Farzadegan H, Polis MA, Wolinsky SM, et al. Loss of human immunodeficiency virus type 1 (HIV-1) antibodies with evidence of viral infection in asymptomatic men. *Ann Intern Med* 1988; 108: 785-90.
13. Abbott MA, Poiesz BJ, Byrne BC, Kwok S, Sninsky JJ, Ehrlich GD. Enzymatic gene amplification: quantitative and qualitative methods for detecting proviral DNA amplified in vitro. *J Infect Dis* 1988; 158: 1158-69.
14. Ou C-Y, Kwok S, Mitchell S, et al. DNA amplification for direct detection of HIV-1 in DNA of peripheral blood mononuclear cells. *Science* 1988; 239: 295-97.
15. Kim S, Byrn R, Grootman J, Baltimore D. Kinetics of HIV gene expression during the one-step multiplication of HIV. In: IV International Conference on Acquired Immunodeficiency Syndrome. Abstracts, vol 1. Stockholm: Swedish Ministry of Health and Social Affairs, 1988: 119.
16. Jaffe HW, Darrow WW, Echenberg DF, et al. The acquired immunodeficiency syndrome in a cohort of gay men. *Ann Intern Med* 1985; 103: 210-14.
17. Seage GR, Horsburgh CR, Hardy AM, et al. Increased suppressor T cells in probable transmitters of human immunodeficiency virus infection. *Am J Publ Health* (in press).
18. Jason J, Holman RC, Dixon G, et al. Effects of exposure to factor concentrates containing donations from identified AIDS patients. *JAMA* 1986; 256: 1758-62.
19. Hausler Jr WJ. Report of the third consensus conference on HIV testing sponsored by the association of state and territorial public health laboratory directors. *Infect Control Hospital Epidemiol* 1988; 9: 345-49.
20. Chang CL. An introduction to stochastic processes and their applications. 2nd ed. New York: Krieger, 1980.
21. Longini IM, Clark WS, Haber M, Horsburgh CR. The stages of HIV infection: waiting times and infectious contact rates. In: Castillo-Chavez C, ed. Mathematical and statistical approaches to AIDS epidemiology. New York: Springer-Verlag (in press).
22. Esteban JI, Shih JW-K, Tai CC, et al. Importance of western blot analysis in predicting infectivity of anti-HTLV III/LAV positive blood. *Lancet* 1985; ii: 1083-86.

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SPECIFICITY OF CALCIUM CHANNEL AUTOANTIBODIES IN LAMBERT-EATON MYASTHENIC SYNDROME

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Summary Autoantibodies that interfere with neurotransmitter release by affecting the function of voltage-operated calcium channels (VOCCs) have been found in patients with Lambert-Eaton myasthenic syndrome (LES). To find out the nature of the antigen to which these autoantibodies bind, tests were done with ω -conotoxin, which blocks some types of VOCCs. LES antibodies were able to immunoprecipitate VOCCs prepared from the human neuronal cell line IMR32 which were pre-labelled with the specific VOCC ligand ω -conotoxin. LES autoantibodies are also able to specifically down-regulate the expression of VOCCs in IMR32 cells. A new radioimmunoassay for the quantitative detection of LES antibodies was developed and found to be of value in distinguishing LES patients from patients with myasthenia gravis and some other neurological disorders.

Introduction

Lambert-Eaton myasthenic syndrome (LES) is a neurological disorder characterised by a reduction in the amount of neurotransmitters released presynaptically.¹⁻⁴ It is often associated with small-cell carcinoma of the lung (SCLC), and also with immunological disorders.^{1,5}

In 1981 Lang et al⁶ suggested that LES was an autoimmune disorder. Since then plasma and IgG from LES patients have been shown to induce in mice the same electrophysiological⁷⁻¹⁰ and morphological^{11,12} alterations found in man.^{3,13} Also, LES autoantibodies can affect the activity of voltage-operated calcium channels (VOCCs) in both human small-cell carcinoma cells^{14,15} and in bovine chromaffin cells.¹⁶ However, nothing is yet known, at the biochemical level, about the nature of the autoantigen

recognised by LES antibodies. In particular, it would be important to know whether these antibodies bind to the calcium channel itself or alter its function indirectly by binding to other membrane molecules.

A new tool for the pharmacological and biochemical characterisation of VOCCs is ω -conotoxin (ω Ctx), a 27-aminoacid peptide synthesised by the marine snail *Comus geographus*.^{17,18} ω -Ctx binds and blocks neuronal VOCCs specifically and thus inhibits the release of several neurotransmitters.¹⁹⁻²² ω -Ctx also blocks VOCCs on SCLC cells¹⁵ but has no effects on the VOCCs of other non-neuronal tissues.^{23,24}

There are high-affinity binding sites for ¹²⁵I- ω Ctx in a human neuroblastoma cell line, IMR-32.²⁵ This toxin blocks part of the VOCCs expressed in IMR-32 cells (the remainder being blocked by dihydropyridines), and it has the valuable property of being an irreversible ligand for the calcium channel. Here we describe an immunoprecipitation assay, similar to the one used for many years for the detection of antinicotinic receptor antibodies in myasthenia gravis patients,²⁶ its diagnostic value in LES, and its use in examining the antigen-specificity of LES autoantibodies.

Patients and Methods

Patients

In the first set of experiments, the subjects were 12 patients with typical clinical and electrophysiological features of LES^{1,2} (5 of whom also had an SCLC; 1 with SCLC but no LES; 7 patients with a definite diagnosis of myasthenia gravis; and 12 healthy, age-matched, volunteers with no evidence of neurological or immunological disorders. Some of the LES patients had organ-specific autoantibodies but only 1 of them had nicotinic acetylcholine receptor antibodies.⁵ All the myasthenia gravis patients had nicotinic receptor antibodies (range 2.8-79.5 nmol/l).

In the subsequent "blind" study, coded serum samples (from Dr V. Lennon, Mayo Clinic, Rochester, Minnesota, USA) had been obtained from 10 LES patients (5 with and 5 without SCLC); 6 SCLC patients without LES; and 12 controls with neurological or immunological disorders (Parkinson's disease, amyotrophic lateral sclerosis, multiple sclerosis, insulin-dependent diabetes mellitus). The code was broken after the assay.

Cell Culture

IMR-32 cells (American type Culture Collection, Rockville, USA) were cultured as previously described²⁷ in 10 cm diameter plastic petri dishes containing minimum essential medium to which were added 10% fetal calf serum, 100 IU/ml penicillin, and 100

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23. Courouce A-M, Bouchardeau F, Julien A-M, Faucher V, Lentz M. Blood transfusion and human immunodeficiency virus (HIV) antigen. *Ann Intern Med* 1988; 108: 771-72.
24. Bowen PA, Lobel SA, Caruna RJ, et al. Transmission of human immunodeficiency virus (HIV) by transplantation: clinical aspects and time course analysis of viral antigenemia and antibody production. *Ann Intern Med* 1988; 108: 46-48.
25. L'age-Stehr J, Schwarz A, Offerman G, et al. HTLV-III infection in kidney transplant recipients. *Lancet* 1985; ii: 1361-62.
26. Kumar P, Pearson JE, Martin DH, et al. Transmission of human immunodeficiency virus by transplantation of a renal allograft, with development of the acquired immunodeficiency syndrome. *Ann Intern Med* 1987; 106: 244-45.
27. Schwarz A, Hoffman F, L'age-Stehr J, Tegzes AM, Offerman G. Human immunodeficiency virus transmission by organ donation. *Transplantation* 1987; 44: 21-24.
28. Tucker J, Ludlam CA, Craig A, et al. HTLV-III infection associated with glandular- fever-like illness in a haemophilic. *Lancet* 1985; i: 585.
29. Stricof RL, Morse DL. HTLV II/LAV seroconversion following a deep intramuscular needlestick injury. *N Engl J Med* 1986; 314: 1115.
30. Anon. Needlestick transmission of HTLV-III from a patient infected in Africa. *Lancet* 1984; ii: 1376-77.
31. Neisson-Vernant C, Arfi S, Mathez D, Liebowitch J, Monplaisir N. Needlestick HIV seroconversion in a nurse. *Lancet* 1986; ii: 814.
32. Oskenhendler E, Harzic M, Le Roux J-M, Rabian C, Clauvel JP. HIV infection with seroconversion after a superficial needlestick injury to the finger. *N Engl J Med* 1986; 315: 582.
33. Wallace MR, Harrison WO. HIV seroconversion with progressive disease in health care worker after needlestick injury. *Lancet* 1988; i: 1454.
34. Marcus RA. National surveillance of health-care workers exposed to the blood of patients infected with the human immunodeficiency virus: update of the Centers for Disease Control's cooperative needlestick surveillance project. *N Engl J Med* 1988; 319: 1118-23.
35. Gioannini P, Sinicco A, Cariti G, et al. HIV infection acquired by a nurse. *Eur J Epidemiol* 1988; 4: 119-20.
36. Centers for Disease Control. Update: human immunodeficiency virus infections in health-care workers exposed to blood of infected patients. *MMWR* 1987; 36: 285-89.
37. Cooper DA, Gold J, Maclean P, et al. Acute AIDS retrovirus infection. *Lancet* 1985; i: 537-40.
38. Gropman JE, Caiazzo T, Thomas MA, et al. Lack of evidence of prolonged HIV infection prior to antibody conversion. *Blood* 1988; 71: 1752-54.
39. Rinaldo C, Kingsley LA, Neumann J, et al. Association of human immunodeficiency virus (HIV) p24 antigenemia with decrease in CD+ lymphocytes and onset of acquired immunodeficiency syndrome during the early phase of HIV infection. *J Clin Microbiol* 1989; 27: 880-84.