CONCISE REPORT

Detection of HIV Antigen and Specific Antibodies to HIV Core and Envelope Proteins in Sera of Patients With HIV Infection

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The sera of well-characterized populations were examined for three markers of human immunodeficiency virus (HIV) infection: HIV antigen (HIV Ag), and antibodies to HIV envelope (gp41) and core (p24) proteins. Of 563 serum samples tested, 251 were from HIV-infected patients diagnosed as having AIDS manifested by opportunistic infections (AIDS-OI), AIDS-associated Kaposi's sarcoma (AIDS-KS), or AIDS-related complex (ARC). One hundred seventy-six specimens tested were from asymptomatic high-risk individuals, and 136 were from heterosexual control subjects or patients with non-AIDS-related disease. None of the 136 control individuals tested had HIV Ag or HIV antibodies to either p24 or gp41. Of the 427 HIV-seropositive individuals, 99% to 100% were positive for gp41 antibodies to HIV. In contrast, the seroprevalence of p24 antibodies to HIV varied from 23% to 83% and appeared to be inversely associated with the severity of the patients' clinical symptoms. When specimens were analyzed for the presence of HIV Ag, in seropositive individuals the prevalence rate for this marker was lowest (1.4%) in asymptomatic individuals and highest (50%) in the AIDS-OI diagnosed group. Also, 240 cases with AIDS-KS, AIDS-OI, and ARC and the group of asymptomatic high-risk individuals were analyzed for T helper/T lymphocytes (T4) cell number and T4/T8 ratio; only one (2.0%) HIV Ag-positive case showed a T4 cell number —400 and a normal T4/T8 ratio. These studies appear to demonstrate a direct correlation between the presence of HIV Ag and the severity of clinical complications of HIV infection.

THE PUTATIVE AIDS human immunodeficiency virus (HIV) has been isolated from peripheral blood mononuclear cells, cerebrospinal fluid, semen, neural tissue, saliva, and tears.1-5 Various serologic tests have been developed to detect the presence of antibodies to HIV in patients with AIDS, AIDS-related complex (ARC), and asymptomatic virus carriers; these include radioimmune precipitation, enzyme-linked immunosorbent assay (ELISA), immunofluorescence, and Western blot procedures.

The availability of recombinant HIV proteins has permitted the development of ELISA techniques for the measurement of specific antibodies to HIV core (p24) and envelope (gp41) proteins in the serum or plasma of infected individuals.1 In addition, methods have been developed to detect viral antigens in tissue culture fluids using solid-phase, HIV-immune Ig as a capture antibody.2 We measured circulating HIV core antigen and antibodies to envelope and core proteins in 563 serum samples, including specimens from patients with AIDS and ARC, and from asymptomatic homosexual individuals at high risk for AIDS, as well as from heterosexual patients with non-AIDS-related diseases and healthy heterosexual control populations.

MATERIALS AND METHODS

Patients and Controls

The sera of 563 individuals, including 251 patients with symptomatic HIV-related disease, 176 asymptomatic high-risk persons, and 136 control subjects were studied. The 251 male patients with HIV-related symptoms included 30 with AIDS manifested by opportunistic infections (AIDS-OI), 142 with AIDS-Kaposi’s sarcoma (AIDS-KS), and 79 with ARC.

The population of asymptomatic high-risk individuals examined included 4 homosexual men and 2 heterosexual women who had direct sexual exposure to patients known to have AIDS, 6 intravenous (IV) drug abusers, 16 previously asymptomatic homosexual men who presented with acute herpes zoster infections (VZV), and 148 asymptomatic “healthy” homosexual men.

Included in the control groups were 51 male and female heterosexual patients with illnesses unrelated to HIV; including 14 with systemic lupus erythematosus, 15 who were seropositive for hepatitis B surface antigen, 12 elderly heterosexual patients with classic KS, 9 heterosexual VZV patients, and 1 undiagnosed healthy HIV-seronegative male patient with unexplained persistent low T helper lymphocyte numbers. Also included as controls were 85 healthy heterosexual laboratory personnel from the New York Medical Center and healthy heterosexual blood donors from the Greater New York Blood Center.

Serum Samples

One-half of the sera samples tested had been frozen and stored at −30°C and thawed just before testing; the remainder of the samples were tested as fresh specimens prior to storage.

HIV Markers

HIV-antigen assay (HTLV-III EIA). The HIV assay evaluated in this study detects primarily the p24 core antigen of HIV as previously described (Abbott Laboratories, N Chicago).9

A neutralization procedure was performed on all repeatedly HIV Ag-positive samples to verify the test results. The HIV antigen in the specimen was neutralized by adding 50 μL of a polyclonal human anti-HIV IgG solution prior to running the HIV Ag assay as described above. Both negative and positive control and positive standard controls were mixed with either buffer or neutralizing solution and were run in parallel with the sample specimens; 200 μL of the standard control or sera specimens together with 50 μL of buffer or neutralizing solution were incubated for 2 hours at room temperature. An HIV antibody-coated bead was then added to each sample, and the HIV Ag assay run as previously described. A

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Submitted February 10, 1987; accepted April 22, 1987.

Supported in part by Abbott Laboratories, the Howard Gilman Foundation, the Aaron Diamond Foundation, and Grants No. CA 19529 and NCI-CA 35982 from the National Institutes of Health, Bethesda, MD.

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0006-4971/87/7002-0036$3.00/0


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positive result was confirmed when a reduction of at least 50% of the recorded OD was observed in samples incubated with the neutralizing solution as compared with the standard control incubated with the buffer.

**HIV anti-core (p24) or anti-envelope (gp41) antibodies.** The competitive immunoassay using recombinant antigens (CIA-RA) was described previously (Abbott Laboratories, N Chicago).9

**Quantitation of T Cell Subsets**

OKT4 and OKT8 monoclonal antibodies were purchased from Ortho Pharmaceutical and were used to determine the proportion of positive cells according to the manufacturer’s instructions using a FACS 4 instrument.

**RESULTS**

**Prevalence of HIV Antigens and Antibodies to HIV**

The prevalence of HIV Ag and of specific antibodies to p24 or gp41 in seropositive members of high-risk groups is shown in Table 1.

None of the healthy heterosexual controls or patients with diseases unrelated to AIDS were positive for any of these markers. From the groups of patients clinically diagnosed as AIDS-OI, AIDS-KS, or ARC, eight samples (3%) were negative for HIV Ag and both HIV antibodies. Thirty-six percent to 50% of the ARC and AIDS patients’ samples that were seropositive (or one with both HIV antibodies) showed detectable amounts of HIV Ag. Asymptomatic subjects who were positive for one or both of the HIV antibodies had the lowest prevalence of HIV Ag (1.4%). Most of them, (83.3%), were positive for antibodies to p24.

The serum marker patterns of circulating HIV-Ag and antibodies were remarkably similar in the ARC and AIDS-KS groups. The small cohort of homosexuals with acute VZV infection showed a much higher prevalence of HIV Ag than did the asymptomatic homosexual group and a pattern of HIV markers resembling the AIDS-KS and ARC patients sera tested. In all three groups, HIV Ag was associated with gp41 (86 of 97) and seldom with p24 (10 of 97). This association was statistically significant ($P < .01$). The prevalence of at least one of the HIV markers in the sera of asymptomatic and asymptomatic homosexuals was 97% and 49%, respectively.

In all the groups tested, the most consistent marker of HIV infection was the antibody to gp41. The frequency of HIV gp41 antibody in AIDS patients with KS was 99%; in patients with OI it was 100%. In asymptomatic individuals, 80% of those with antibodies to gp41 also had antibodies to p24. The comparable figures for patients with ARC was 51%, for AIDS-KS 50%, and for AIDS-OI only 23%. The prevalence of p24 antibody in patients with AIDS-OI was significantly lower than that of the ARC and AIDS-KS groups and much lower than that of asymptomatic individuals positive for antibody to gp41 ($P < .001$).

Table 2 shows the patterns of HIV markers found in HIV-infected subjects from the various risk groups studied. All three HIV markers, HIV Ag, gp41 antibody, and p24 antibody, occurred together infrequently in all groups. A single ARC patient had only HIV Ag, with no evidence of gp41 or p24 antibodies. In all groups except AIDS-OI, the most prevalent pattern observed was the presence of both HIV p24 and gp41 antibodies, in the absence of HIV Ag. A high percentage of HIV-seropositive individuals had antibody only to gp41 and not to p24. None of the sera contained anti-p24 antibody in the absence of anti-gp41 antibody.

**Relationship Between Number of T4(CD4) Cells or T4/T8 Ratio and Presence of HIV Ag, and Antibodies to HIV**

Blood from 240 persons was analyzed for the number of T4 lymphocytes and the T4/T8 ratio. In 63 of these, the blood was negative for both antibodies to HIV and for HIV Ag. Data in Table 3 show that among 177 seropositive persons, only one in whom HIV Ag was detected had >400 T4 cells/$\mu$L or a ratio of T4/T8 >1. In the remaining 49 HIV Ag-positive individuals, the T4 number was <400/$\mu$L ($P < .001$).

**DISCUSSION**

Diagnosis of HIV infection presently relies on either the detection of specific IgG antibodies by various methods or on the isolation of the virus from cultured lymphocytes. Current methods of culturing HIV involve the cocultivation of stimulated patients’ cells with normal lymphocytes in vitro. This method may lead to the activation of integrated or otherwise latent HIV. As a consequence, current techniques of culturing HIV may not distinguish between infections in which HIV is actively replicating and those in which the virus is latent. The ability to detect circulating HIV Ag in sera, as demonstrated in this study, may circumvent this problem in many patients, since it is likely that the presence of detectable circulating viral antigen in the serum requires replicating virus.10 In addition, correlation of the clinical stages of

### Table 1. Prevalence of HIV Infection and of HIV Markers in High-Risk Groups and Controls

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of Subjects</th>
<th>No. Seropositive (%)</th>
<th>Prevalence (%) of Marker in HIV Seropositives</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>HIV Ag</td>
</tr>
<tr>
<td>AIDS-OI</td>
<td>30</td>
<td>30 (100)</td>
<td>50.0</td>
</tr>
<tr>
<td>AIDS-KS</td>
<td>142</td>
<td>141 (99.3)</td>
<td>39.7</td>
</tr>
<tr>
<td>ARC</td>
<td>79</td>
<td>72 (91.1)</td>
<td>43.6</td>
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<tr>
<td>Exposed subjects</td>
<td>12</td>
<td>10 (83.3)</td>
<td>80.0</td>
</tr>
<tr>
<td>HZV homosexual</td>
<td>16</td>
<td>1.5 (93.8)</td>
<td>26.6</td>
</tr>
<tr>
<td>Asymptomatic homosexual</td>
<td>148</td>
<td>72 (48.6)</td>
<td>1.4</td>
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<td>Control/unrelated disease</td>
<td>51</td>
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</tr>
<tr>
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the infections with circulating HIV core antigen and with antibodies against core and envelope proteins may provide further insight into the immunobiology of this disease.

We examined 563 sera by means of ELISA techniques for circulating core antigen and antibodies against gp41 and p24. The data demonstrate that the prevalence of circulating antigen increased with increasing severity of the clinical syndromes associated with HIV. This observation is consistent with the idea that increasing amounts of viral expression occur in the patient as the disease progresses, despite a fall in the number of lymphocytes expressing CD4 (T4 cells), the cell in which most viral replication is believed to occur. The increasing frequency of viral antigen expression as the disease progresses could result either from the production of large amounts of p24 by the remaining CD4 cells or from other types of cells known to support the replication of HIV.

In our series, only one patient (with ARC) had circulating antigen but no detectable antibody to HIV.* Asymptomatic individuals have been described from whom HIV was isolated even though they were seronegative.15 This observation is most easily explained by assuming that viremia occurs at least transiently after infection, prior to the development of detectable antibodies. In studies in which sequential samples of serum were examined, the presence of HIV Ag in high-risk populations was demonstrated prior to the development of IgG antibodies to HIV.16 A transient antigenemia may occur in some individuals soon after infection, and the presence of persistent circulating antigen may be associated with symptomatic disease.

The pattern of antigen and antibodies in otherwise asymptomatic homosexual men who have herpes zoster are similar to those seen in patients with ARC, suggesting a biological similarity between these two modes of clinical presentation of HIV infection.11

The prevalence of antibodies to gp41, coded by the env gene and antibodies to the core protein p24 also differ in patients with different stages of infection with HIV. All asymptomatic and symptomatic seropositive individuals had antibody to gp41. No persons in this study were detected with antibody to p24 but without antibody to gp41. The data presented do not enable us to determine whether this reflects the nature of the population groups studied or whether the ELISA test used is more sensitive in detecting antibodies against gp41 than p24, as has been suggested by a comparison of this method with immunoblots.7 The prevalence, in seropositive individuals, of antibodies to the core protein p24 decreased from 80% in asymptomatic individuals to 50% in patients with ARC or AIDS-KS and to 23% in patients with AIDS-OI. A sequential loss of detectable antibody to p24 with the onset of symptoms has previously been reported in one adult and one child.12

Several possibilities exist that might explain this decrease in antibodies to p24 in patients with more advanced disease. Theoretically, individuals who make antibodies to HIV core as well as envelope proteins after infection may be less likely to develop progressive, symptomatic disease. The step-wise decrease in prevalence of this HIV antibody in patients with AIDS-KS and patients with AIDS-OI, coupled with the frequent development of OI by patients with AIDS-KS makes this explanation less attractive. The ability to make detectable amounts of antibody against p24 may decline as the disease progresses.

In addition, the increased frequency of circulating HIV core protein may, in part, interfere with the ability to detect anti-p24 antibody in patients with more advanced disease, as has been suggested.12 It is evident, however, from the patterns of antigen and the two antibodies shown in Table 2, that in some specimens both core antigen and antibody to p24 were detected.

The finding that circulating HIV core protein correlates with the severity of the clinical stage of disease resulting from HIV infection is supported by the fact that the presence of core antigen also correlates with the severity of the host's immunologic defect as assessed by depressed numbers of T4 cells (Table 2). In these patients, the low number of T4 cells in turn is correlated with symptomatic disease, as would be expected. The data on T4 cells also confirm that in the groups of patients studied circulating p24 core antigen was present most frequently in individuals with low numbers of circulating T4 lymphocytes. Although HIV preferentially

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*The serum sample was repeatedly negative for antibody to HIV; however, a serum sample drawn on the same patient 3 days later contained antibody to gp41 by the same ELISA assay and also contained p24 antigen.
binds to and infects cells bearing the T4 (CD4) surface marker, the increased presence of core antigen in patients with low numbers of T4 lymphocytes in the blood suggests that the expression of HIV infection may be occurring in other types of cells or in T4 lymphocytes that are not circulating.

The data indicate that the development of p24 antigenemia is associated with a loss of antibodies against p24, a low number of T4 lymphocytes, symptomatic disease, and a poor prognosis.

ACKNOWLEDGMENT

We thank Dr Saul Krugman for providing the control samples. We also thank Mitchell Speer for his administrative assistance and Tom Nichols for his excellent secretarial assistance.

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