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Detection of p24 Antigen with and without Immune Complex Dissociation for Longitudinal Monitoring of Human Immunodeficiency Virus Type 1 Infection

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Sequential specimens obtained from 87 multicenter AIDS cohort study participants were tested by three p24 antigen tests. They included a polyclonal enzyme immunoassay (EIA), a monoclonal EIA, and a monoclonal EIA after immune complex dissociation (ICD) of specimens. Subjects were grouped into two categories defined by real-time testing with the polyclonal EIA: 39 had become positive for p24 antigen (antigen converters) during follow-up, and 48 had progressed to AIDS without detectable antigenemia. Twenty-four (61%) antigen converters were positive by ICD-monoclonal EIA about 1 year earlier than by monoclonal EIA. In contrast, only 12 (25%) patients who progressed to AIDS without detectable antigenemia became positive by ICD-p24 EIA before developing AIDS. Thus, the main benefit of ICD treatment may be to detect p24 antigenemia approximately 1 year before the regular assay rather than to identify additional antigenemic people. Qualitative plasma RNA levels were also determined in longitudinal samples from 20 antigen converters and 7 men who developed AIDS without antigenemia. Although mean human immunodeficiency virus type 1 RNA levels were higher in antigen-positive than in antigen-negative samples (P < 0.002), more than half (11 of 20) of the antigen converters had no measurable change in human immunodeficiency virus type 1 RNA associated with change to antigen positivity.

Human immunodeficiency virus type 1 (HIV-1) p24 antigen is an indirect marker of viral replication and has been shown to correlate with disease progression (4, 6, 14, 16, 18). In addition, the detection of p24 antigen can be used as a criterion for entry into clinical trials and subsequently for monitoring antiviral therapy (8, 20, 21). Various studies have shown that treating serum samples with acid (9, 11) or base (19) can dissociate p24 antigen complexes with anti-p24 antibodies and thus increase the rate of positivity for p24 antigen. In addition, immune complex disruption (ICD) treatment can be helpful for the evaluation of antiviral therapy in HIV-infected adults (2) and may contribute to the diagnosis of infection in infants (17). The utility of this procedure in longitudinal follow-up of seropositive individuals at various stages of disease, however, has not been established.

We determined the utility of acid treatment for detecting p24 antigen in sequential samples obtained from 87 participants in a multicenter AIDS cohort study in Chicago, Ill. The participants consisted of patients who evolved from negative to positive for p24 antigen (p24 converters), most of whom progressed to AIDS within 2 years on average, and patients who developed AIDS but were never positive for p24 antigen. We also studied the relationship between detection of p24 antigen with and without ICD treatment and plasma HIV-1 RNA levels measured by semiquantitative PCR amplification.

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MATERIALS AND METHODS

Patients and specimens. The multicenter AIDS cohort was assembled in 1984 to 1985 to study the natural history of HIV-1 infection (10). All participants have a physical evaluation and blood drawn every 6 months. We selected sequential plasma specimens (n = 368) from two groups of subjects. The first group consisted of 39 men who had evolved from negative to positive for p24 antigen (p24 converters), as measured by the polyclonal p24 enzyme immunoassay (EIA, Abbott Laboratories, N. Chicago, Ill.) during follow-up. For each subject, sequential specimens consisted of the first sample positive by polyclonal p24 EIA and 4 to 10 samples preceding it, for a total of 215 samples. The second group consisted of 48 patients who had all progressed to clinical AIDS without becoming p24 antigen positive. For each subject, sequential specimens consisted of the sample obtained closest to AIDS diagnosis and 1 to 5 samples preceding it, for a total of 153 samples.

Of the 39 antigen converters, 29 had developed AIDS, on average, 1.6 years (range, 0.1 to 5.75 years) after becoming positive for p24 antigen. Of the remaining ten p24 converters, six were still free of clinical AIDS 2.5 to 7.5 years later, three were lost to follow-up at 1, 3, and 4 years after p24 antigen was detected, and one died of causes unrelated to HIV infection 3.5 years later.

Seventeen subjects had received antiretroviral therapy. Eight antigen converters had started treatment (zidovudine, did, or ddC) before a mean of 1.5 years (range, 0.4 to 1.8 years) prior to becoming positive for p24 antigen. Of the remaining ten p24 converters, six were still free of clinical AIDS 2.5 to 7.5 years later, three were lost to follow-up at 1, 3, and 4 years after p24 antigen was detected, and one died of causes unrelated to HIV infection 3.5 years later.

Seventeen subjects had received antiretroviral therapy. Eight antigen converters had started treatment (zidovudine, did, or ddC) before a mean of 1.5 years (range, 0.1 to 4 years) before AIDS diagnosis. Five subjects had initiated therapy at the time of the first p24 antigen-positive result (n = 1) or AIDS diagnosis (n = 4). The remaining 70 subjects had not received antiretroviral treatment.

Testing for p24 antigen. All samples were tested in parallel by polyclonal p24 EIA, monoclonal p24 EIA, and ICD-monoclonal p24 EIA (Abbott Laboratories). Acid treatment of specimens was performed essentially as previously described (9). Briefly, 100 μl of serum was mixed with 190 μl of 0.15 M glycine (pH 2.0), and the mixture was heated at 70°C for 10 min. After the mixture was cooled to room temperature, 10 μl of 3.5 M Tris base (pH 10.8) was added to neutralize the sample, and a 200-μl aliquot was tested per the manufacturer’s instructions. All reactive samples were confirmed by neutralization. The cutoff of each assay was calculated by adding 0.05 to the mean optical density of three negative controls.

Plasma RNA measurement. Detection of virion-associated RNA in 50 μl of plasma was obtained by immunoassay with anti-gp120/41-coated microparticles, direct lysis, and reverse transcription-amplification as previously described (5). HIV-1 RNA was measured in the same aliquots that were tested for p24 antigen. Semiquantitation of RNA levels was obtained by comparison with log10 dilutions of a calibrated external plasma standard, corresponding to approximately 103, 104, and 105 copies of HIV-1 RNA per ml of plasma. The absolute
sensitivity of the assay was approximately 100 copies of HIV-1 RNA or 1.000 virions per ml (50 virions per 50 µl), as determined by blind evaluation of serial dilutions of cell-free virus added to seronegative plasma and quantitated by electron microscopy (13).

RESULTS

Detection of p24 antigen. Among antigen converters, p24 antigen was detected in 39 (16.7%) specimens by p24 polyclonal EIA, in 65 (30.2%) specimens by p24 monoclonal EIA, and in 126 (58.6%) specimens by ICD-p24 monoclonal EIA. Of the 39 samples positive by polyclonal EIA, one could not be tested by the monoclonal antibody-based assays because of insufficient volume. Of the 38 remaining samples, 36 were positive by monoclonal EIA and 37 were positive by ICD-p24 monoclonal EIA. Of the 65 specimens positive by p24 monoclonal EIA, 61 were positive by ICD-p24 monoclonal EIA, 2 were negative, and 2 were borderline (15% below cutoff). Once p24 antigen was detected by monoclonal antibody-based assays, subjects usually remained positive throughout follow-up. In only six subjects was early positivity by p24 monoclonal EIA (n = 3) or ICD-p24 monoclonal EIA (n = 3) followed by transient negativity. Three of those six transient decreases coincided with initiation of therapy.

Among subjects who progressed to AIDS without antigenemia, no specimen was reactive by p24 polyclonal EIA, 11 were reactive by p24 monoclonal EIA, and 36 (23.5%) were positive by ICD-p24 monoclonal EIA. One of the two specimens that were positive by p24 monoclonal EIA was negative by ICD-p24 monoclonal EIA.

The detection of p24 antigen by each test as a function of time in each group of subjects is presented in Fig. 1 and 2. Among antigen converters (Fig. 1), the p24 monoclonal EIA identified antigenemia a mean of 0.97 year (range, 0.5 to 2.5 years) earlier than the polyclonal assay in 19 (48%) subjects (Fig. 1B). About one-half (10 of 19) of these subjects were positive by monoclonal EIA 6 months earlier than by polyclonal EIA. The ICD-p24 monoclonal EIA detected antigenemia a mean of 1.37 years (range, 0.5 to 3.0 years) earlier than the polyclonal assay in 35 (89%) subjects (Fig. 1C) and a mean of 1.29 years (range, 0.5 to 3.0 years) earlier than the p24 monoclonal EIA in 24 (61%) subjects (Fig. 1D). Among the 24 subjects for whom p24 antigen was detected earlier by ICD-monoclonal than monoclonal EIA, 11 were positive only 6 months earlier.

Among subjects who progressed to AIDS without detectable antigenemia by the polyclonal EIA, the p24 monoclonal EIA identified p24 antigen 7 months before AIDS in only one subject (Fig. 2B). The ICD-p24 monoclonal EIA detected antigenemia a mean of 1.54 years (range, 0.1 to 6.0 years) before AIDS diagnosis in 12 (25%) subjects (Fig. 2C). In an additional three subjects, p24 antigen was detected by ICD-p24 monoclonal EIA at the time of AIDS diagnosis.

Plasma RNA levels. For each of 20 antigen converters, three types of longitudinal specimens were selected and tested for plasma HIV-1 RNA. They consisted of specimens negative for p24 antigen, positive only by ICD-monoclonal EIA, and positive by monoclonal (and ICD-monoclonal) EIA. Overall, 65 of 71 samples (91%) had at least 10^3 copies of HIV-1 RNA per ml. Figure 3A represents the HIV-1 RNA levels in each category of samples from antigen converters. Mean HIV-1 RNA levels were higher in p24 antigen-positive (10^3.95 copies per ml) than in p24 antigen-negative (10^3.05 copies per ml) samples (P = 0.002; Fisher’s exact test). Interestingly, more than half (11 of 20) of the subjects did not have significant changes in HIV-1 RNA level when they evolved from antigen-negative to ICD-p24 antigen positive (Fig. 3A).

For seven persistently antigen-negative subjects, specimens collected within 2 years before AIDS diagnosis were analyzed. The mean HIV-1 RNA level was 10^3.0 copies per ml. In the year preceding AIDS diagnosis, five subjects had no change in HIV-1 RNA levels (Fig. 3B). Two patients, including one who evolved from antigen negative to ICD-p24 antigen positive, had 10-fold increases in HIV-1 RNA level.

DISCUSSION

The detection of HIV-1 p24 antigen is a good prognostic marker of progression to AIDS. Individuals who are positive for p24 antigen have a risk of developing AIDS over the following 2 to 3 years about six times higher than those who remain antigen negative (6, 14, 16). Treatments such as ICD...
increase the rate of positivity for p24 antigen compared with the regular assay (2, 9, 11). This could identify more people at risk of progressing to AIDS or allow detection of p24 antigen earlier than with the regular EIA in antigenemic patients or both.

Our results document the stepwise increment in sensitivity measured by polyclonal, monoclonal, and ICD-monoclonal EIAs. The monoclonal EIA detected p24 antigen about 1 year earlier than the polyclonal EIA in 28% of antigen converters but was not significantly more sensitive in persistent antigen-negative subjects. Compared with the monoclonal EIA, ICD treatment allowed detection of antigenemia about 1.5 years earlier in 61% of antigen converters and 1.5 years before AIDS diagnosis in 28% of persistently antigen-negative subjects. This indicates that ICD treatment is primarily useful for earlier detection of p24 antigen in patients who become antigenemic before developing AIDS.

The relationship between detection of p24 antigen and plasma viral load as measured by RNA detection was investigated for each group of subjects. Surprisingly, at least half of the antigen converters progressed from ICD-p24 antigen negative to positive without a detectable change in plasma RNA level. This could reflect differences in quantitative power of the two methods since the HIV-1 RNA PCR measurement, unlike the p24 antigen, can only discriminate 10-fold changes. On the other hand, these results are consistent with previous reports that some of the p24 antigen detected is not associated with intact virions (3, 7) and that addition of Triton X-100, which could increase p24 antigen detection by lysing intact virions, does not significantly affect the results of the p24 EIA (1). Thus, detection of p24 antigen may sometimes reflect a defective immune response which can no longer effectively complex or destroy viral proteins rather than increasing viral replication. Detection of plasma HIV-1 RNA could, therefore, more accurately reflect viral replication.

Our study found a relatively high proportion of men who progressed to AIDS without detectable p24 antigen, even by ICD-p24 EIA. Of 220 cohort participants who have developed AIDS, 75 (34%) had no detectable p24 antigen by the polyclonal EIA. Of 48 patients who could be analyzed, 12 (25%) became positive by ICD-p24 monoclonal EIA before developing AIDS. If we extrapolate these results to the entire group of antigen-negative progressors, perhaps 18 (25%) of 75 antigen-negative progressors would become positive by ICD-p24 EIA before developing AIDS. The remaining 57 subjects, which represents 26% (57 of 220) of all progressors, would remain negative for p24 antigen. Since each virion contains approximately 1,000 molecules of p24 antigen and two copies of HIV-1 RNA (12), the antigen-negative subjects should also have 10,000 or fewer copies of HIV-1 RNA per ml of plasma (12).
This is consistent with our measurement of a mean $10^3$ copies of HIV-1 RNA per ml in these subjects. These results indicate that a sizable proportion (~25%) of HIV-infected individuals can progress to AIDS despite having relatively low steady-state levels of circulating virus in peripheral blood. In these cases, determinants such as the genetic background, the efficacy of the immune response, and other host factors (15) may be more relevant to disease progression than plasma viremia.

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