Antigen Detection for Human Immunodeficiency Virus

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INTRODUCTION

The human immunodeficiency virus (HIV) was first isolated by F. Barre-Sinoussi and her colleagues at the Pasteur Institute in Paris in 1983 (6). The isolation procedure involved coculture of lymph node cells from a symptomatic person with phytohemagglutinin-stimulated, peripheral blood mononuclear cells from a healthy donor. The presence of a retrovirus, first designated lymphadenopathy-associated virus and later officially called HIV, in culture was suggested by the appearance of high levels of reverse transcriptase (RT) in culture supernatants after only 7 days and was confirmed by electron microscopy. Scientists at the National Cancer Institute in the United States further developed the culture method to permit continuous production of HIV in a human T-cell lymphoma cell line (H9) (56). This procedure made available large amounts of HIV antigen necessary for development and production of serological test reagents. The initial HIV serological tests became indispensable scientific tools to define the extent of the HIV infection epidemic (9). Further, their use in screening blood donors has virtually eliminated blood transfusion as a route of HIV transmission (71). Several test formats are now in use for the detection of anti-HIV antibody, including enzyme immunoassay (EIA) (10, 58), Western blot (immunoblot) (15), indirect immunofluorescence assay (11), and radioimmuno-precipitation (40). Recently, more rapid, simple, and inexpensive tests for anti-HIV antibody have been developed for use in developing countries (12, 72).

Sero logical analysis of HIV-infected individuals has, until recently, been limited to the detection of anti-HIV antibodies. A new development is the direct detection of HIV antigen(s). The first methods described detected HIV antigen in cell culture supernatants, thus providing immunological confirmation of RT assay results (33, 48). The HIV antigen assays have been further refined to detect directly HIV antigens in body fluids from infected individuals (30, 52, 74). The HIV antigen assay has become an important laboratory test for diagnosis, prognosis, and research on HIV infection. The intent of this review is to describe (i) the historical development of the HIV antigen assay and (ii) a technical comparison of selected commercially available assays and (iii) their clinical and research applications.

DEVELOPMENT OF HIV ANTIGEN ASSAYS

Assays specific for HIV antigen were first described as adjuncts to HIV purification and characterization methods (4, 13). A competition radioimmunoassay for HIV p24 was developed to determine the antigenic cross-reactivity among HIV type 1, human T-cell leukemia virus type I (HTLV-I), and HTLV-II p24 proteins isolated from culture supernatants (62).

Later, indirect immunofluorescence assay methods, using human anti-HIV antibodies to detect infected cells, were also developed (46). The initial indirect immunofluorescence assay methods for detecting infected cells in culture were technically difficult and required extensive absorption of the human polyclonal antibodies to prevent nonspecific binding to cultured lymphocytes (46). Immunofluorescence assays with anti-HIV polyclonal or monoclonal antibodies were also used to detect the distribution of HIV in blood and tissue (17, 55, 56, 67, 68, 73).

HIV antigen EIA s were first developed to facilitate detection of HIV in culture. Early methods for viral assay in cell culture were restricted to detection of RT activity (29, 60, 64), with electron microscopy studies used as confirmation (6, 46). The routine use of RT as a laboratory test presented several disadvantages (22). Since all human retroviruses possess functionally identical RTs, the assay cannot discriminate among HIV and other retroviruses possibly present. Furthermore, the RT assay is technically very complex, requires a radioisotopic label, and cannot be conveniently automated, although a micromethod has now been described (63).

The first HIV antigen EIA was described by Mc Douglas et al. in 1985 (48). High-titered anti-HIV immunoglobulin G (IgG) purified from a seropositive individual was used in a sandwich EIA test format. The IgG was bound to the plastic surface of a microtiter well as the capture antibody. HIV culture supernatant was added, followed, after incubation and washing, by incubation with an enzyme-labeled human anti-HIV antibody. If antigen was present, a "sandwich" formed among the capture antibody, HIV antigen, and
enzyme-labeled detector or probe antibody. After the addition of enzyme substrate, a positive reaction resulted in color development. The sensitivity of the assay compared favorably with the RT assay, with the added advantage that 20-fold less culture supernatant was needed, thus facilitating the miniaturization of cultures from flasks to cell culture tubes.

In another early assay, two noncompeting monoclonal antibodies with specificity for the major HIV core protein, p24, were used with a rabbit polyclonal anti-HIV antibody in various combinations as capture and probe reagents to detect HIV in culture (33). This assay was as sensitive as RT for the detection of HIV in culture. The p24 monoclonal antibodies were the first described that differentiated between HIV strains on the basis of reactivities to core proteins. Both monoclonal antibodies (MAb) were produced against HIV (HTLV-III) but one, MAb 22-6, reacted with the prototype strains HIV (HTLV-III), HIV (lymphadenopathy-associated virus type 1), and HIV (acquired immunodeficiency syndrome [AIDS]-related virus type 2), while the other, MAB 22-3, reacted only with HIV (HTLV-III) and HIV (lymphadenopathy-associated virus type 1) and not with HIV (AIDS-related virus type 2). By altering the test format, e.g., using either MAB 22-6 or MAB 22-3 as capture antibody with the rabbit anti-HIV antibody as probe, it was possible to identify several antigenically distinct HIV strains from the same patient (7).

These initial tests detected HIV antigens only in virus culture supernatants; the sensitivities were not adequate for the detection of the very low titers of HIV antigen present in body fluids of most HIV-infected individuals. The development of a triple-antibody sandwich procedure, using antibodies primarily specific for the p24 antigen, provided the needed amplification to detect HIV antigens in clinical specimens (30, 74). The p24 antigen is a predominant structural protein for HIV and may be present in higher concentrations than other viral antigens in clinical specimens. For example, a system composed of human anti-HIV antibody, rabbit anti-HIV p24, and enzyme-labeled goat anti-rabbit antibody detected between 50 and 100 pg of whole HIV or purified p24 per ml, respectively, 1,000 times more sensitive than the RT assay (74).

**COMMERCIAL HIV ANTIGEN ASSAYS**

Several HIV antigen EIAs are now commercially available. Table 1 describes the specific methods for five such EIAs test kits: Abbott Laboratories (North Chicago, Ill.), Du Pont Co. (Wilmington, Del.), Coulter Electronics, Inc. (Hialeah, Fla.), Genetic Systems Inc. (Seattle, Wash.), and Cellular Products Inc. (Buffalo, N.Y.). All assays use a solid-phase antigen capture matrix composed of either polyclonal or monoclonal anti-HIV antibodies, bound to either polystyrene beads or microtiter plate wells. Test samples are incubated with the capture matrix. After incubation and washing to remove unbound protein, the matrix is incubated with anti-HIV probe antibody reagent. If HIV antigen has bound to the capture matrix during the first incubation, the HIV-specific antibody in the probe reagent will also bind, thus forming an immobilized, three-component, antibody-antigen-antibody sandwich. Use of capture and probe antibodies with different specificities ensures that both probe and capture antibody will not compete for the same antigen moieties. A color development, or “indicator,” step is performed next. The commercial antigen EIAs vary most substantially in this step. Four of the assays, Du Pont, Abbott, Coulter, and Cellular Products, use an enzyme-conjugated indicator reagent that specifically binds to the immobilized sandwich. Genetic Systems consolidates the indicator and probe reagent into a single step by using an enzyme-conjugated anti-HIV antibody as probe reagent. The enzyme used in all assays is horseradish peroxidase.

Three assays, Du Pont, Coulter, and Cellular Products, use biotinylated probe antibody. In these three assays, the indicator reagent is composed of horseradish peroxidase-conjugated streptavidin. The four assays that use a horseradish peroxidase-conjugated indicator reagent require an additional incubation step in which specific binding occurs between the immobilized sandwich and the horseradish peroxidase-conjugated indicator. Following a series of washes to remove unbound conjugate, the final assay step for all five tests is the addition of an enzyme substrate reagent. Substrate turnover produces color that is proportional to the amount of HIV antigen present. Spectrophotometric absorbance measurements can thus be related to antigen concentration.

All kits incorporate a method for qualitative determination of results, based on a comparison of sample absorbances with a “negative cutoff” or “reactive threshold” value. The reactive threshold value is derived from the mean of replicate absorbance values observed for a negative control material supplied with the kit. Any sample with an absorbance exceeding the reactive threshold value is considered reactive. Semiquantitative results may be obtained with

<table>
<thead>
<tr>
<th>Manufacturer of assay</th>
<th>Capture step</th>
<th>Detection step</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Antibody</td>
<td>Matrix</td>
</tr>
<tr>
<td>Abbott</td>
<td>H anti-HIV</td>
<td>Polystyrene beads</td>
</tr>
<tr>
<td>Coulter</td>
<td>mc anti-p24</td>
<td>Microtiter well</td>
</tr>
<tr>
<td>Du Pont</td>
<td>pcR anti-p24</td>
<td>Microtiter well</td>
</tr>
<tr>
<td>Genetic Systems</td>
<td>mc anti-p24</td>
<td>Microtiter well</td>
</tr>
<tr>
<td>Cellular Products</td>
<td>H anti-HIV</td>
<td>Microtiter well</td>
</tr>
</tbody>
</table>

*H anti-HIV, human anti-HIV; R anti-HIV, rabbit anti-HIV; mc anti-p24, monoclonal anti-p24; G anti-R, goat anti-rabbit; pcR anti-p24, polyclonal rabbit anti-p24; HRPO, horseradish peroxidase; Str-HRPO, streptavidin-horseradish peroxidase; OPD, o-phenylenediamine; TMB, tetramethylbenzidine; S, serum; P, plasma; CCS, cell culture supernatant.

* Lowest recommended dilution of stock standard material.

* Rapid qualitative method.

TABLE 1. Comparison of selected commercial HIV antigen assays

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CLIN. MICROBIOL. REV.

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### Table 1—Continued

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Conjugate</th>
<th>Conjugate time (min)</th>
<th>Conjugate temp (°C)</th>
<th>Substrate</th>
<th>Incubation</th>
<th>Data analysis</th>
<th>Confirmation neutralization assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>G anti-R</td>
<td>HRPO</td>
<td>120</td>
<td>40</td>
<td>OPD</td>
<td>30</td>
<td>22</td>
<td>Semiquantitative (15 pg/ml)</td>
</tr>
<tr>
<td>Str-HRPO</td>
<td>30</td>
<td>37</td>
<td>OPD</td>
<td>30</td>
<td>37</td>
<td>Quantitative</td>
<td>No (repeat)</td>
</tr>
<tr>
<td>Str-HRPO</td>
<td>30</td>
<td>37</td>
<td>TMB</td>
<td>30</td>
<td>22</td>
<td>Semiquantitative</td>
<td>No (repeat)</td>
</tr>
<tr>
<td>Str-HRPO</td>
<td>15</td>
<td>37</td>
<td>TMB</td>
<td>30</td>
<td>25</td>
<td>Quantitative</td>
<td>No</td>
</tr>
</tbody>
</table>

Abbott and Genetic Systems assays, using an optional standard curve procedure. Serial dilutions of a stock standard preparation are run in parallel with the control and experimental sera. A standard curve is derived by plotting absorbance data for the standard dilutions against the dilution factor, using graphical or linear/quadratic least-squares procedures. Comparison of the experimental absorbance with the standard curve allows semiquantitative determination of antigen concentration. The method is described as "semiquantitative," because the absolute antigen concentration in the stock standard preparation is not stated in molar or concentration units, but in arbitrary units per millilitre, presumably standardized among succeeding test kit lots. Three test kits, Du Pont, Coulter, and Cellular Products, provide a quantitative method for determination of p24 antigen concentration. A stock curve is prepared by using serial dilutions of a stock solution containing p24 antigen at a known concentration. Standardization between manufacturers for HIV antigen quantitation has not yet been achieved as evidenced by a recent report in which Abbott standards used in the Du Pont assay were 4-fold lower than stated values and Du Pont standards in the Abbott test were 2.5- to 4-fold higher than stated values (S. Crowe, N. McManus, M. McGrath, and J. Mills, Abstr. 4th Int. Conf. AIDS 1988, vol. 2, abstr. 1616, p. 79). These discrepancies may reflect differences in the avidity and specificity of both capture and probe antibodies as well as in the material used as the standard.

Reactive specimens should be confirmed. The preferred method of confirmation uses an antigen neutralization assay provided with some kits. This procedure requires preincubation of the sample with antibody to HIV, followed by repetition of the antigen EIA. HIV antigen present in the sample will be sequestered in immune complexes during the preincubation step and consequently will not be bound during the capture phase of the antigen EIA. This results in a reduction in measured absorbance for the neutralized specimen in comparison with that of a non-neutralized identical sample run simultaneously. Initially reactive specimens are considered confirmed when an absorbance reduction of at least 50% is observed for the neutralized sample and an absorbance exceeding the reactive threshold value is observed for the non-neutralized sample.

Specimen requirements are similar for all commercial antigen EIAs. Serum, plasma, and cell culture supernatants may all be tested. The use of ethylenediaminetetraacetic acid, sodium citrate, or heparin as anticoagulant does not appear to affect performance. All manufacturers discourage testing of turbid, lipemic, or hemolyzed samples. Specimen storage at 2 to 8°C for up to 1 week and extended frozen storage at −20 or −70°C are both permissible, although repeated freezing and thawing is not recommended.

To achieve maximum sensitivity, three assays, Du Pont, Abbott, and Genetic Systems, require an overnight incubation period for the capture phase. Completion of the probe and indicator phases generally requires an additional 2 to 5 h, establishing a realistic turnaround time of approximately 24 h for the complete assay. An additional 24 h is needed to complete confirmatory neutralization or repeat procedures. Thus, for most kits, a confirmed positive result would require at least 2 days, using the most sensitive test protocol. The Coulter and Cellular Products quantitative assays require only 1- and 2-h incubations, respectively, at 37°C during the capture phase, thus considerably shortening the turnaround time. Incubation times for the remaining steps are equivalent to those of the other assays. Du Pont and Genetic Systems both offer a rapid procedure for qualitative determination that allows completion of the assay in 5 and 2.5 h, respectively.

Based on list price, the cost per bead or well for the four assays are as follows: Abbott, $8.00 per bead; Du Pont, $4.79 per well; Genetic Systems, $6.00 per well; Coulter, $5.50 per well; and Cellular Products, $2.35 per well. The cost per reportable test will be considerably higher, since account must be taken of standards, controls, replicate samples, and confirmatory determinations on initially reactive specimens.

Sensitivity limits for the four assays detailed in Table 1 vary between 10 and 30 pg/ml when controls are tested. However, comparison of relative sensitivity is difficult due to the diversity of antigenic materials used for controls, different antibody specificities, and lack of a uniform reference standard. Several reports have compared the sensitivity of a select group of commercial assays, but the available published data are not sufficient to draw conclusions about the relative performance of all commercially available HIV antigen EIAs (34, 36; Crowe et al., Abstr. 4th Int. Conf. AIDS 1988; F. Barin, A. Couroucle, A. Maniez, and M. Rouziouz, Abstr. 4th Int. Conf. AIDS 1988, vol. 2, abstr. 1618, p. 80).

The HIV antigen EIAs are very specific. Nonspecific reactivity has not been demonstrated with a large group of human viruses, including HTLV-I, cytomegalovirus, herpes simplex virus (30); poliovirus type 1, adenovirus type 5, varicella-zoster virus (22); and numerous cell culture lines. However, limited cross-reactivity for HIV type 2 has been reported (D. Paul, M. Knugge, M. Kennedy, D. Mack, and J. Leibowitch, Abstr. 4th Int. Conf. AIDS 1988, vol. 2, abstr. 1647, p. 87). Clinical evaluation of the Abbott HIV antigen assay in large low-risk populations has demonstrated great specificity. False-positive results were not detected in >250,000 blood donors when the neutralization test was used to confirm repeatedly reactive tests (U. Backer, F. Weinauer, A. G. Gathof, E. Gossrau, J. Eberle, and F.
Deinhardt, Abstr. 4th Int. Conf. AIDS 1988, vol. 2, abstr. 7757, p. 364). The repeatedly reactive rate of 0.18% was entirely attributable to false-positive results as confirmed by using the confirmatory assay, the demonstration of HIV antigen. New HIV-infected individuals were not identified by HIV antigen screening in this low-risk population, indicating that the prevalence of HIV infection may be too low to warrant the addition of this test as a routine screening procedure at blood donor centers.

If it is assumed that all seropositive individuals are infected with HIV, the sensitivity of the HIV antigen assay in detecting HIV infection directly in patient specimens varies from 4% in asymptomatic seropositive subjects to 70% in AIDS patients (38). Of 22 seropositive adult and pediatric patients with AIDS, 19 had detectable amounts of antigen in their sera, in contrast to 1 of 13 asymptomatic seropositive individuals (30). Antigen positivity rates in cerebrospinal fluid demonstrated a similar trend, being 55% and 0%, respectively, for AIDS and asymptomatic patients.

The apparent lack of sensitivity of the HIV antigen assay in detecting HIV infection, particularly in asymptomatic individuals, limits its use as a screening assay for HIV infection in low-risk groups, but there are other useful clinical and research applications for this test. Further studies may better define the sensitivity, specificity, and clinical utility of this new type of laboratory test. It will also be especially important to establish quality assurance procedures to control intra- and interlaboratory proficiency in the performance of the HIV antigen EIAs (40; F. B. Hollinger, J. W. M. Gold, L. Meyer, C. van de Horst, R. Makuch, W. Werks, and the NIH-NIAID AIDS Program, Treatment Branch, Abstr. 4th Int. Conf. AIDS 1988, vol. 2, abstr. 1618, p. 82).

CLINICAL LABORATORY APPLICATIONS

Infection with HIV may result in an acute viral syndrome that mimics influenza or mononucleosis with headache, fever, and malaise (1). After the acute phase, during which seroconversion may occur, an asymptomatic period of variable duration follows. Eventually, after a mean latent period of about 8 years, virtually all HIV-infected individuals develop laboratory and clinical signs of HIV disease, with progression to AIDS as the ultimate outcome (50).

The laboratory diagnosis of HIV infection is routinely based on the use of anti-HIV serological tests (9) and virus isolation (46). Other laboratory markers for HIV disease progression are nonspecific and include enumeration of T cells, serum immunoglobulin, and β₂-microglobulin levels (50, 75). The HIV antigen assay has improved the laboratory diagnosis of HIV infection by providing a more sensitive marker than routine antibody screening during the early stages of infection and for confirming neonatal infection in the presence of passively transferred maternal anti-HIV IgG (49). In addition, HIV antigen assays may be useful in the diagnosis of persistent infection in children who become seronegative but who remain infected (8). The HIV antigen assay has also improved diagnosis of HIV infection by culture methods by eliminating the need for the RT assay and by increasing the test sensitivity and decreasing the time for results (22).

The detection of HIV antigen in serum, plasma, or cerebrospinal fluid after seroconversion is a useful indicator of HIV disease progression, offering a simple and more specific alternative to the nonspecific laboratory tests. The HIV antigen assay can also be used to monitor the efficacy of antiviral drug therapy (14, 19, 35, 59).

Diagnosis of Early HIV Infections

HIV antigen has been detected in serum or plasma in the early stages of HIV infection even before seroconversion in a small percentage of infected asymptomatic subjects (3, 30) and in those persons with an acute HIV-associated illness (27, 39, 69, 70). However, shortly after seroconversion, most HIV-infected individuals become HIV antigen negative or are only transiently positive (69).

The HIV antigen assay should be very useful for the diagnosis of acute HIV infection. However, the general benefit of this assay for screening asymptomatic high-risk or low-risk populations will depend on the length of the “window” period between infection and seroconversion and on the incidence of infection in the population tested. For example, the HIV antigen assays will be less useful if the window is short and the incidence of infection is low.

Several studies have attempted to determine the length of the window phase. In a cohort of 741 homosexual men, 35 seroconverted during the study period and HIV antigen was detected in 14% before and in 17% after seroconversion (30). The elapsed time between antigen detection and seroconversion was 3 months, and the antigen titer fell below detectable limits within 1 month of seroconversion. Other reported time intervals between infection and seroconversion vary between 1 and 5 months (3, 39), while one recent report suggests a longer window period (57).

Diagnosis of HIV Infection in Infants

Standard serological diagnosis of HIV infection in high-risk neonates and young children is difficult due to the passive transfer of maternal anti-HIV IgG (49) and the presence of virus in the absence of autologous anti-HIV antibodies following the loss of maternal antibody (8). Classically, the detection of specific IgM antibodies has proved useful in differentiating between the specific response of the infant to an infection and passively transferred maternal antibody (25). However, in the case of HIV infection, IgM assays have not been helpful (26). A recently developed and potentially better assay for detecting neonatal antibody measures in vitro production of HIV-specific antibody by peripheral blood lymphocytes in culture (5). Currently, HIV culture and HIV antigen assay are the most specific diagnostic tests for HIV infection in neonates and young children (8, 49).

Since maternal anti-HIV antibody may be transferred to the fetus in utero without the concomitant transfer of HIV (49), the early detection of anti-HIV antibody from peripheral blood is not a definitive diagnosis for HIV infection of the fetus or neonate. Detectable titers of maternal antibody can persist for a considerable period. Among 71 neonates born to seropositive mothers, the median age for disappearance of maternal anti-HIV antibody was 11 months; 75% of all children lost antibody by 1 year (49). When HIV antigen assay was applied to samples from 20 of the seropositive children, only 55% were positive, confirming infection. Presumably, the other 45% were false-positive antibody assays due to the presence of residual maternal antibody. In another study, HIV antigen assay was positive when serum from each of six seropositive pediatric AIDS patients was tested (30).

The incidence of HIV-infected neonates and young children who remain seronegative for extended periods is much higher than in the adult population. The HIV antigen EIA has particular value in confirming HIV infection in these
groups. Of 85 children with HIV infection, ranging in age from 2.5 months to 4 years, 9 (11%) were seronegative by anti-HIV EIA (8). However, all nine were positive by HIV antigen EIA. By using Western blot and a more sensitive EIA with recombinant antigens, antibody was detected in four of nine of these children, indicating that only five were truly antibody negative. Lange and Goudsmit (42) also reported on one of nine HIV-infected children with antigenemia and absent anti-HIV antibody titer.

In conclusion, detection of HIV antigens may be useful in the specific diagnosis of HIV infections in infants with maternal anti-HIV IgG. When used in combination with more sensitive anti-HIV antibody tests, HIV antigen tests may confirm HIV infection in seronegative children born to seropositive mothers.

HIV Antigen Detection in Virus Culture

Initially, isolation of HIV in culture was the only method for the diagnosis of HIV infection. The development of HIV antibody tests (9) soon supplanted culture for screening. Results of early efforts to use HIV culture methods to assess antiviral drug efficacy were ambiguous. HIV isolation studies failed to provide endpoints that predicted therapeutic efficacy in zidovudine (azidothymidine) drug trials because the method lacked sufficient sensitivity to detect the subtle inhibitory effect of the drug (23). As more effective drugs are developed, however, HIV isolation may prove to be more useful for testing them. At present, virus isolation is most useful for the diagnosis of infection in neonates and for the identification of new HIV strains.

Virus culture has not been widely used because the procedure is difficult. Fresh human peripheral blood mononuclear cells are the most sensitive target cells and are not easily obtained. Most important, however, is the need for frequent RT assays to detect HIV in culture. The use of RT as a marker for HIV production suffers from (i) technical difficulty, (ii) lack of specificity (i.e., it cannot distinguish among retroviruses), and (iii) long incubation time, requiring 1 to 3 weeks of cocultivation of lymphocytes from the patient with phytohemagglutinin-stimulated normal lymphoblasts for the detection of HIV (22).

Recently, HIV antigen assays have been compared with RT assay for the detection of HIV in culture (22, 37, 44, 51, 68, 74). The majority of reports have shown that the HIV antigen assay is more sensitive than RT, yielding positive results on more specimens and at an earlier time in the culture process. In one study (22), RT activity first appeared in 34 cultures 6 to 16 days after initiation of culture. The average time for detection of HIV by RT was 9.6 days. In contrast, p24 was detectable in culture supernatants by HIV antigen assay within 3 to 9 days, with an average detection time of 5.9 days.

The relative sensitivity of HIV antigen EIA is 100- to 1,000-fold greater than that of the RT assay in detecting virus in cell culture supernatants (22, 32, 51, 74). If performance standards and reproducibility (41) can be established for this test, it offers a suitable substitute for RT in HIV culture applications.

HIV Antigen as a Predictive Marker

A strong correlation has been established between the onset of antigenemia and the progression of disease after seroconversion (2, 19, 24, 29, 30, 38, 42, 45, 47, 50, 53, 61). This suggests the use of HIV antigen EIA as a prognostic index in assessing disease progression. In a group of 96 hemophiliacs, a much higher incidence of AIDS was observed in those individuals who were positive for HIV antigen (2). Moreover, antigen titers tended to increase with disease progression. In eight individuals who initially tested antigen positive, the antigen titer increased almost threefold over approximately 3 years and all patients developed clinical complications of active HIV infection. Another study of 32 patients with AIDS-related complex (ARC) found a significant correlation between HIV antigen positivity or absence of anti-p24 antibody or both and progression to AIDS (38). Within a 23-month period, 23% of ARC patients who were initially antigen positive progressed to AIDS, whereas only 8% of those who were initially antigen negative did so. Of 34 seropositive men, 8 of 16 with antigenemia developed AIDS or ARC, compared with only 1 of 18 without antigenemia (53).

In a recent study of 281 men who were seropositive at entry, 46 (16%) were initially HIV antigen positive (50). Over the next 3 years, 59% of men initially positive for HIV antigen progressed to AIDS versus 15% of those who were initially HIV antigen negative. The presence of p24 antigen was positively correlated with reduced T-helper lymphocyte levels (T4 < 400 x 10^6/liter) as a predictive variable. Furthermore, HIV antigen was a better predictor of disease progression than loss of anti-p24 antibody. Other useful predictors of disease progression were elevated serum levels of β₂-microglobulin, packed cell volume of <40, proportion of T4 lymphocytes of <25%, and absolute number of lymphocytes being ≥200 x 10^3/liter. The implication of this study is that simple and more straightforward laboratory tests such as HIV antigen EIA, β₂-microglobulin, and packed cell volume may be as useful as the more difficult T-cell assays for the prediction of HIV disease progression.

HIV antigen test results may also serve as a useful marker for predicting the efficacy of antiviral drug therapy. A correlation has been established between reduction in antigenemia in zidovudine-treated HIV antigen-positive subjects and resultant beneficial clinical outcome (34, 23). HIV antigen detection has demonstrated significant reduction in antigen levels in serum, plasma, and cerebrospinal fluid of subjects treated with zidovudine (14, 17, 18, 35, 59). In drug efficacy studies, zidovudine has been compared with acyclovir (18) and suramin (20), and only zidovudine was effective in reducing antigenemia.

The HIV antigen assay is an effective laboratory tool for assessing antiviral drug activity in clinical studies. One potential limitation is the low prevalence of antigenemia observed in asymptomatic, HIV-infected populations (4, 30, 38), which may limit the use of the HIV antigen assay to drug studies involving ARC and AIDS patients in whom the prevalence of detectable antigenemia is much higher. Even more sensitive HIV antigen assays need to be developed or tests measuring other parameters, such as β₂-microglobulin levels, may prove to be important adjuncts to this type of study (50, 75; M. Jacobson, D. Abrams, J. Wilber, P. Volberding, J. Mills, R. Chaisson, and A. Moss, Abstr. 4th Int. Conf. AIDS 1988, vol. 2, abstr. 3646, p. 178).

Research Laboratory Applications

antigen assays is their requirement for much smaller volumes of test sample as compared with the RT assay. This has made it possible to miniaturize the culture system, allowing testing of multiple replicate samples for calculating 50% tissue culture infectious dose of the HIV preparation used for inocula. Because the HIV antigen assays are optimized for the detection of p24 (a conserved region of the HIV genome), an endpoint of infectivity may be determined for different HIV isolates in any susceptible cell line or in fresh peripheral blood mononuclear cells. Assays in which infectivity endpoints are established by HIV antigen assay have been used to detect antibody neutralization of infectivity and inactivation of HIV by both chemical and physical methods.

Caution should be exercised when using HIV antigen assays in biosassays for HIV inactivation because residual noninfectious inactivated HIV may be detected in the test inoculum (Keddie et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1987). Therefore, an increase in HIV antigen titer over time, indicating active replication, must be detected in cultures to confirm the presence of virus replication following inactivation procedures.

**BIOLOGY OF HIV ANTIGENEMIA**

The biology of HIV antigen expression and modulation of anti-p24 antibody titers during infection can be described by two possible models. The first would propose that, after infection, HIV begins replication in the host and expression of virus is detected before seroconversion as HIV antigen in peripheral blood or cerebrospinal fluid. Then, due to undetermined host or virus factors, HIV becomes latent or expressed at negligible levels until later in the disease course, when replication is again activated and viral protein titers increase with formation of immune complexes that lead to depletion of antibody titers, particularly anti-p24.

Another model proposes that continuous low-level production of HIV occurs during the incubation period following primary infection (3). The reduction in initial antigenemia following seroconversion is caused by immune complex formation that continues during the seropositive asymptomatic phase of the infection. Progression to ARC or AIDS is associated with an increase in antigen production, eventually leading to a condition of antigen excess, resulting in antigenemia and reduction of p24 antibody titer.

Differentiating between the two alternatives will require answering two key unresolved questions: (i) what causes rapid loss of antigenemia following seroconversion; and (ii) what initiates the antigenemia with disease progression?

The onset of antigenemia following primary infection occurs within approximately 1 to 5 months. Following seroconversion, the HIV antigen level quickly falls, usually to undetectable levels, where it remains in clinically asymptomatic seropositive individuals. Onset of ARC or AIDS may be accompanied by detection of antigenemia. A steadily increasing antigen titer and decrease in anti-p24 antibody titer have been strongly correlated with a progression of disease and poor prognosis (43, 50).

In support of the latent HIV model, Von Sydow et al. (69) observed HIV antigen-containing immune complexes during the phase of decreasing antigen titer immediately following seroconversion. However, no complexed antigen was detected for months following loss of detectable antigenemia, suggesting low or absent synthesis of HIV in the host. Others (42, 52), however, have reported contrasting results, indicating the presence of antigen-antibody complexes in infected individuals with or without the presence of anti-p24 antibodies, although immune complexes were more commonly found in those subjects without detectable anti-p24 levels (42). Further studies are necessary to resolve the question of whether reduced viral production or masking of antigen by antibodies is primarily responsible for the loss of antigenemia following seroconversion.

Several lines of evidence support the view that, during late infection, the reappearance of antigenemia and decrease in anti-p24 antibody are related to an increase in viral replication, rather than selective inhibition of antibody production. It is difficult to envision an immune regulatory mechanism that selectively inhibits only one among the several antibodies to HIV produced. Furthermore, it has been observed that some patients with high antigen levels also lack antibody to p55, the undegraded gag precursor polypeptide from which p24 is ultimately processed (2, 28). Thus, the reduction in antibody appears not to be totally selective for anti-p24. Preliminary studies also suggest that an increased synthesis of p24 antigen is related to increased HIV replication, since a strong correlation exists between plasma HIV antigen titer and the ability of plasma to infect normal peripheral blood lymphocytes in vitro with HIV (21). Correlation of HIV antigen detection and polymerase chain reaction for HIV ribonucleic and deoxyribonucleic acids may help to resolve these questions raised about the biology of HIV antigenemia (31).

**CONCLUSIONS**

Development of EIA procedures for the direct determination of HIV antigen has been of significant benefit in both clinical and research arenas. The commercial availability of HIV antigen assays has permitted HIV antigen determinations to be performed on a routine basis.

The HIV antigen EIA is an effective substitute for the more costly and complex RT assay for detecting HIV infection in cell culture, and it has facilitated the development of microculture methods. Wider implementation of HIV antigen EIA for in vitro virus identification should result in more widely available, less costly, and more rapid culture determinations. The use of simple and straightforward laboratory tests such as β₂-microglobulin and packed cell volume, in conjunction with HIV antigen EIA, may be as useful as the more difficult T-cell assays for predicting HIV disease progression.

In clinical applications, the predictive value of HIV antigen EIA testing of serum or plasma is highly dependent on the prevalence of HIV infection in the target human population. In low-prevalence populations (e.g., blood donors), the routine use of HIV antigen EIA as a screening procedure does not appear to be justified. In high-risk asymptomatic populations, the HIV antigen EIA should prove increasingly useful in diagnostic and prognostic applications, which include (i) early detection of infection in seronegative individuals who are members of a high-risk group; (ii) confirmation of HIV infection in children, in whom the prevalence of seronegative HIV-infected individuals may be higher than in the adult population; (iii) diagnosis of HIV infection in neonates of seropositive mothers; (iv) use of prognostic index during the later stages of infection; and (v) assessment of therapeutic efficacies of various antiviral drugs.

The interrelationship between HIV antigen and anti-HIV antibody titers during disease progression appears to be quite complex. Disappearance of initial antigenemia following seroconversion and later reappearance, frequently accompanied by a reduction in anti-p24 antibody titer, may be
explained by at least two different models. One proposes that varying levels of viral expression modulate antigen titers, while the other proposes that selective suppression of anti-p24 antibody during late infection results in reappearance of antigenemia. Currently available studies offer conflicting evidence in support of both models, and definitive conclusions must await further studies.

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LITERATURE CITED


