At least three other forms of cell-mediated immunity to HIV have been described: non-cytolytic CD8+ T cell–mediated suppression of HIV replication, ADCC, and NK cell activity. *Non-cytolytic CD8+ T cell–mediated suppression of HIV replication* refers to the ability of CD8+ T cells from an HIV-infected patient to inhibit the replication of HIV in tissue culture without killing infected targets. There is no requirement for HLA compatibility between the CD8+ T cells and the HIV-infected cells. This effector mechanism is thus nonspecific and appears to be mediated by soluble factor(s) including the CC-chemokines RANTES (CCL5), MIP-1α (CCL3), and MIP-1β (CCL4). These CC-chemokines are potent suppressors of HIV replication and operate at least in part via blockade of the HIV co-receptor (*CCR5*) for R5 (macrophage-tropic) strains of HIV-1 (see above). *ADCC*, as described above in relation to humoral immunity, involves the killing of HIV-expressing cells by NK cells armed with specific antibodies directed against HIV antigens. Finally, *NK cells* alone have been shown to be capable of killing HIV-infected target cells in tissue culture. This primitive cytotoxic mechanism of host defense is directed toward nonspecific surveillance for neoplastic transformation and viral infection through recognition of altered class I MHC molecules.

DIAGNOSIS AND LABORATORY MONITORING OF HIV INFECTION

The establishment of HIV as the causative agent of AIDS and related syndromes early in 1984 was followed by the rapid development of sensitive screening tests for HIV infection. By March 1985, blood donors in the United States were routinely screened for antibodies to HIV. In 1996, blood banks in the United States added the p24 antigen capture assay to the screening process to help identify the rare infected individuals who were donating blood in the time (up to 3 months) between infection and the development of antibodies. In 2002, the ability to detect early infection with HIV was further enhanced by the licensure of nucleic acid testing (NAT) as a routine part of blood donor screening. These refinements decreased the interval between infection and detection (window period) from 22 days for antibody testing to 16 days with p24 antigen testing and subsequently to 12 days with NAT. The development of sensitive assays for monitoring levels of plasma viremia ushered in a new era of being able to monitor the progression of HIV disease more closely. Utilization of these tests, coupled with the measurement of levels of CD4+ T lymphocytes in peripheral blood, is essential in the management of patients with HIV infection.

DIAGNOSIS OF HIV INFECTION

The CDC has recommended that screening for HIV infection be performed as a matter of routine health care. The diagnosis of HIV infection depends on the demonstration of antibodies to HIV and/or the direct detection of HIV or one of its components. As noted above, antibodies to HIV generally appear in the circulation 3–12 weeks following infection.

The standard blood screening tests for HIV infection are based on the detection of antibodies to HIV. A common platform is the ELISA, also referred to as an *enzyme immunoassay* (EIA). This solid-phase assay is an extremely good screening test with a sensitivity of >99.5%. Most diagnostic laboratories use commercial kits that contain antigens from both HIV-1 and HIV-2 and thus are able to detect antibodies to either. These kits use both natural and recombinant antigens and are continuously updated to increase their sensitivity to newly discovered species, such as group O viruses (Fig. 226-1). The fourth-generation EIA tests combine detection of antibodies to HIV with detection of the p24 antigen of HIV. EIA tests are generally scored as positive (highly reactive), negative (nonreactive), or indeterminate (partially reactive). While the EIA is an extremely sensitive test, it is not optimal with regard to specificity. This is particularly true in studies of low-risk individuals, such as volunteer blood donors. In this latter population, only 10% of EIA-positive individuals are subsequently confirmed to have HIV infection. Among the factors associated with false-positive EIA tests are antibodies to class II antigens (such as may be seen following pregnancy, blood transfusion, or transplantation), autoantibodies, hepatic disease, recent influenza vaccination, and acute viral infections. For these reasons, anyone suspected of having HIV infection based on a positive or inconclusive EIA result should ideally have the result confirmed with a more specific assay such as the Western blot. One can estimate whether an individual has a recent infection with HIV-1 by comparing the results on a standard EIA test that will score positive for all infected individuals with the results on an assay modified to be less sensitive ("detuned assay") that will score positive for individuals with established HIV infection and negative for individuals with recent infection. In rare instances, an HIV-infected individual treated early in the course of infection may revert to a negative EIA. This does *not* indicate clearing of infection; rather, it signifies levels of ongoing exposure to virus or viral proteins insufficient to maintain a measurable antibody response. When these individuals have discontinued therapy, viruses and antibodies have reappeared.

The most commonly used confirmatory test is the Western blot **(Fig. 226-29)**. This assay takes advantage of the fact that multiple HIV antigens of different, well-characterized molecular weights elicit the production of specific antibodies. These antigens can be separated on the basis of molecular weight, and antibodies to each component can be detected as discrete bands on the Western blot. A negative Western blot is one in which no bands are present at molecular weights corresponding to HIV gene products. In a patient with a positive or indeterminate EIA and a negative Western blot, one can conclude with certainty that the EIA reactivity was a false positive. On the other hand, a Western blot demonstrating antibodies to products of all three of the major genes of HIV (*gag*, *pol*, and *env*) is conclusive evidence of infection with HIV. Criteria established by the U.S. Food and Drug Administration (FDA) in 1993 for a positive Western blot state that a result is considered positive if antibodies exist to two of the three HIV proteins: p24, gp41, and gp120/160. Using these criteria, ~10% of all blood donors deemed positive for HIV-1 infection lacked an antibody band to the *pol* gene product p31. Some 50% of these blood donors were subsequently found to be false positives. Thus, the absence of the p31 band should increase the suspicion that one may be dealing with a false-positive test result. In this setting it is prudent to obtain additional confirmation with an RNA-based test for HIV-1 and/or a follow-up Western blot. By definition, Western blot patterns of reactivity that do not fall into the positive or negative categories are considered "indeterminate." There are two possible explanations for an indeterminate Western blot result. The most likely explanation in a low-risk individual is that the patient being tested has antibodies that cross-react with one of the proteins of HIV. The most common patterns of cross-reactivity are antibodies that react with p24 and/or p55. The least likely explanation in this setting is that the individual is infected with HIV and is in the process of mounting a classic antibody response. In either instance, the Western blot should be repeated in 1 month to determine whether the indeterminate pattern is a pattern in evolution. In addition, one may attempt to confirm a diagnosis of HIV infection with the p24 antigen capture assay or one of the tests for HIV RNA (discussed below). While the Western blot is an excellent confirmatory test for HIV infection in patients with a positive or indeterminate EIA, it is a poor screening test. Among individuals with a negative EIA and PCR for HIV, 20–30% may show one or more bands on Western blot. While these bands are usually faint and represent cross-reactivity, their presence creates a situation in which other diagnostic modalities (such as DNA PCR, RT-PCR, or p24 antigen capture) must be employed to ensure that the bands do not indicate early HIV infection.

A guideline for the use of these serologic tests in attempting to make a diagnosis of HIV infection is depicted in **Fig. 226-30**. In patients in whom HIV infection is suspected, the appropriate initial test is the EIA. If the result is negative, unless there is strong reason to suspect early HIV infection (as in a patient exposed within the previous 3 months), the diagnosis is ruled out and retesting should be performed only as clinically indicated. If the EIA is indeterminate or positive, the test should be repeated. If the repeat is negative on two occasions, one can assume that the initial positive reading was due to a technical error in the performance of the assay and that the patient is negative. If the repeat is indeterminate or positive, one should proceed to the HIV-1 Western blot. If the Western blot is positive, the diagnosis is HIV-1