of tissue culture cells growing on a coverslip, viral infection can be detected by staining with a monoclonal antibody to a specific viral protein expressed early in viral replication. Thus, virus-infected cells can be detected within hours or days of inoculation, whereas several rounds of infection would be required to produce visible cytopathic effects.

Isolation of virus in tissue culture also depends on the collection of specimens from appropriate sites and the rapid transport of these specimens in appropriate medium to the virology laboratory (Chap. 150e). Rapid transport maintains viral viability and limits bacterial and fungal overgrowth. Enveloped viruses are generally more sensitive to freezing and thawing than nonenveloped viruses. The most appropriate site for culture depends on the pathogenesis of the virus in question. Nasopharyngeal, tracheal, or endobronchial aspirates are most appropriate for the identification of respiratory viruses. Sputum cultures generally are less appropriate because bacterial contamination and viscosity threaten tissue-culture cell viability. Aspirates of vesicular fluid are useful for isolation of HSV and VZV. Nasopharyngeal aspirates and stool specimens may be useful when the patient has fever and a rash and an enteroviral infection is suspected. Adenoviruses can be cultured from the urine of patients with hemorrhagic cystitis. CMV can frequently be isolated from cultures of urine or buffy coat. Biopsy material can be effectively cultured when viruses infect major organs, as in HSV encephalitis or adenovirus pneumonia.

The isolation of a virus does not necessarily establish disease causality. Viruses can persistently or intermittently colonize normal human mucosal surfaces. Saliva can be positive for herpesviruses, and normal urine samples can be positive for CMV. Isolations from blood, cerebrospinal fluid (CSF), or tissue are more often diagnostic of significant viral infection.

Another method aimed at increasing the speed of viral diagnosis is direct testing for antigen or cytopathic effects. Virus-infected cells from the patient may be detected by staining with virus-specific monoclonal antibodies. For example, epithelial cells obtained by nasopharyngeal aspiration can be stained with a variety of specific monoclonal antibodies to identify the specific infecting respiratory virus. Antigen and serologic assays can be multiplexed to detect multiple analytes simultaneously by coupling of reagents to color-coded beads for each analyte and detection by flow cytometry.

Nucleic acid amplification techniques bring speed, sensitivity, and specificity to diagnostic virology. The ability to directly amplify minute amounts of viral nucleic acids in specimens means that detection no longer depends on viable virus and its replication. For example, amplification and detection of HSV nucleic acids in the CSF of patients with HSV encephalitis is a more sensitive detection method than culture of virus from CSF. The extreme sensitivity of these tests can be a problem, because subclinical infection or contamination can lead to false-positive results. Detection of viral nucleic acids does not necessarily indicate virus-induced disease.

Measurement of the amount of viral RNA or DNA in peripheral blood is an important means for determining whether a patient is at increased risk for virus-induced disease and for evaluating clinical responses to antiviral chemotherapy. Nucleic acid technologies for RNA quantification are routinely used in AIDS patients to evaluate responses to antiviral agents and to detect viral resistance or noncompliance with therapy. Virus-load measurements are also useful for evaluating the treatment of patients with HBV and HCV infections. Nucleic acid testing or direct staining with CMV-specific monoclonal antibodies to quantitate virus-infected cells in the peripheral blood (CMV antigenemia) is useful for identifying immunosuppressed patients who may be at risk for CMV-induced disease.

DRUG TREATMENT FOR VIRAL INFECTIONS

Multiple steps in the life cycles of viruses can be effectively targeted by antiviral drugs (Chaps. 215e and 216). Nucleoside and nonnucleoside reverse transcriptase inhibitors prevent HIV provirus synthesis, whereas protease inhibitors block maturation of the HIV and HCV polyprotein after infection of the cell. Enfuvirtide is a small peptide derived from HIV gp41 that acts before cell infection by preventing a conformational change required for initial fusion of the virus with the cell membrane. Raltegravir is an integrase inhibitor that is approved for use with other anti-HIV drugs. Amantadine and rimantadine inhibit the influenza M2 protein, preventing release of viral RNA early during infection, whereas zanamivir and oseltamivir inhibit the influenza neuraminidase, which is necessary for the efficient release of mature virions from infected cells.

Viral genomes can evolve resistance to drugs by mutation and selection, by recombination with a drug-resistant virus, or (in the case of influenza virus and other segmented RNA viral genomes) by reassortment. The emergence of drug-resistant strains can limit the efficacy of antiviral therapy. As in antibacterial therapy, excessive and inappropriate use of antiviral therapy can select for the emergence of drug-resistant strains. HIV genotyping is a rapid method for identifying drug-resistant viruses. Resistance to reverse transcriptase or protease inhibitors has been associated with specific mutations in the reverse transcriptase or protease genes. Identification of these mutations by polymerase chain reaction amplification and nucleic acid sequencing can be clinically useful for determining which antiviral agents may still be effective. Drug resistance also can arise in herpesviruses but is a less common clinical problem.

IMMUNIZATION FOR THE PREVENTION OF VIRAL INFECTIONS

Viral vaccines are among the outstanding accomplishments of medical science. Smallpox has been eradicated except as a potential weapon of biological warfare or bioterrorism (Chap. 261e). Poliovirus eradication may soon follow. Measles can be contained or eliminated. Excess mortality due to influenza virus epidemics can be prevented, and the threat of influenza pandemics can be decreased by contemporary killed or live attenuated influenza vaccines. Mumps, rubella, and chickenpox are well controlled by childhood vaccination in the developed world. Reimmunization of mature adults can be used to control herpes zoster. New rotavirus vaccines can have a major impact on this leading cause of gastroenteritis and prominent cause of childhood death worldwide. Widespread HBV vaccination has dramatically lowered the frequency of acute and chronic hepatitis and is expected to lead to a dramatic decrease in the incidence of hepatocellular carcinoma. The HPV vaccine was the first vaccine specifically licensed to prevent virus-induced cancer. Use of purified proteins, genetically engineered live-virus vaccines, and recombinant DNA–based strategies will make it possible to immunize against severe infections with other viruses. The development of effective HIV and HCV vaccines is complicated by the high mutation rate of viral RNA polymerase and reverse transcriptase, the population-based and individual divergence of HIV or HCV genomes, and repeated high-level exposure in some populations. Concerns about the use of smallpox and other viruses as weapons necessitate maintenance of immunity to agents that are not encountered naturally.

VIRUSES AS NOVEL THERAPEUTIC TOOLS OR AGENTS

Viruses are being used experimentally to deliver biotherapeutic agents or novel vaccines. Foreign genes can be inserted into viral nucleic acids, and the recombinant virus vectors can be used to infect the patient or the patient's cells ex vivo. Retrovirus integration into the cell genome has been used to functionally replace the abnormal gene in T cells of patients with severe combined immunodeficiency, thereby restoring immune function. Recombinant adenovirus, AAV, and retroviruses are being explored for use in diseases due to single-gene defects, such as cystic fibrosis and hemophilia. AAV carrying a lipoprotein lipase gene is now being used in Europe to treat a rare lipid-processing disease and is the first gene therapy approved for clinical use. Recombinant poxviruses, adenoviruses, and influenza viruses are also being used experimentally as vaccine vectors. Viral vectors are being tested experimentally for the expression of cytokines that can enhance immunity against tumor cells or for the expression of proteins that can increase the sensitivity of tumor cells to chemotherapy. HSV deficient for replication in resting cells is being used to selectively kill proliferating glioblastoma cells after injections into CNS tumors. For improved safety, nonreplicating viruses are frequently used in clinical trials. Potential adverse events associated with virus-mediated gene transfer include the induction of inflammatory and antiviral immune responses. Instances of retrovirus-induced human malignances have raised concerns about the safety of retroviral gene therapy vectors.