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CHAPTER 6

Pathogenesis: Virus-Induced Changes in Cells

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Four of the previous five chapters have been concerned with viruses as such, and the other one (Chapter 3) with their cultivation and assay, which are prerequisites for such investigations. The next group of seven chapters deals with the interactions of viruses with the animals that they infect. However, virus-induced changes at the subcellular and molecular levels are best studied in cultured cells; observations at this level can then be used to interpret changes found in whole animals. Viral cytopathology is as complex as cell biology itself, hence it is not surprising that the subject is still largely at the descriptive level of understanding. The analysis of viral replication has been simplified at a biochemical level by the concept of strategies of viral replication (see Chapter 4); there is as yet no such unifying theme as to how DNA or RNA viruses redirect cellular metabolism and kill or transform infected cells.

The various types of interactions that can occur between virus and cell are summarized in Table 6-1. Viruses may be categorized as cytocidal (lytic) and noncytocidal (nonlytic). Not all infections, whether cytocidal or
TABLE 6-1
*Virus–Cell Interactions*

| Type of infection       | Effect on cell                                                                 | Production of infectious virions                                                                 |
|-------------------------|-------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|
| Cytocidal (lytic)       | Morphological changes in cells (cytopathic effects); inhibition of protein, RNA, and DNA synthesis; cell death | Usually; but cytopathology can occur in nonproductive (abortive) infections                       |
| Persistent noncytocidal | No cytopathic effect; little metabolic disturbance; cells continue to grow and divide; may be associated with loss of the special functions of differentiated cells | Always, although often with reduced yield                                                        |
| (productive)            |                                                                                   |                                                                                                  |
| Persistent noncytocidal | Usually nil                                                                      | Nil. Viral genome persists as episome or integrated. Normally virus is not expressed but may be induced by cocultivation with permissive cells, irradiation, or chemical mutagens |
| (nonproductive)         |                                                                                   |                                                                                                  |
| Transformation          | Alteration in cell morphology; cells survive and can be passaged indefinitely; transformed cells may produce tumors in experimental animals | Rarely by oncogenic DNA viruses and usually by oncogenic RNA viruses                              |

noncytocidal, necessarily lead to the production of new virions. Cell changes of a profound nature, leading to cell death in some cases and cell transformation in others, may also occur in nonproductive (abortive) infections. Looked at from the point of view of the cell rather than the virus, certain kinds of cells are permissive, i.e., they support complete replication of a particular virus, while others are nonpermissive, i.e., replication is blocked at some point. Cytopathic changes can occur in both productive and nonproductive infections and in permissive and nonpermissive cells.

**CYTOPATHIC EFFECTS OF VIRUSES**

The gross and histological appearance of the damage produced in cultured cells by particular cytocidal viruses, known as the *cytopathic*...
effect, is an important diagnostic criterion, since many viruses cause a characteristic cytopathic effect in cells commonly used in diagnostic laboratories (see Chapter 13). Many biochemical changes occur in cells infected with cytocidal viruses. Early in infection, virus-specified proteins often shut down the cell's protein synthesis, an event which is incompatible with its survival. Late in infection, large numbers of viral macromolecules accumulate; some of these, particularly certain capsid proteins, are directly toxic. Viral proteins or virions themselves are sometimes found in large crystalline aggregates or inclusions that visibly distort the cell. Viral proteins are often inserted into the plasma membrane, sometimes quite early in the replication cycle. There are also changes in membrane permeability, leading to osmotic swelling.

Thus there are numerous changes in the virus-infected cell which, individually or cumulatively, can be lethal. Cell damage by certain viruses can occur even without replication of the virus, e.g., when late stages of the expression of the viral genome are blocked, as in infection of alveolar macrophages by influenza or parainfluenza viruses.

**Shutdown of Cellular Protein Synthesis**

Most cytocidal viruses code for proteins that shut down the synthesis of cellular proteins; cellular RNA and DNA synthesis are usually affected secondarily. The shutdown is particularly rapid and severe in infections of cultured cells by picornaviruses, some poxviruses, and herpesviruses, all of which are rapidly cytopathogenic. With other viruses (e.g., adenoviruses) the shutdown is later and more gradual, while with noncytocidal viruses such as arenaviruses and retroviruses there is, by definition, no shutdown and no cell death. Some viruses (e.g., flaviviruses) are cytocidal, even though they do not shut down cellular protein synthesis very well, indicating that this is not the only mechanism involved.

**Cytopathic Effects of Viral Proteins**

Although toxin production is not a feature of viral infections, viral capsid proteins, e.g., adenovirus penton and fiber proteins, in high concentrations are often toxic and may be a principal cause of a cytopathic effect. Pathological changes in cells may follow the accumulation of viral proteins late in the replication cycle after infection at low multiplicity, or may be seen quite early, as a laboratory artifact, after the experimental use of very large inocula.

**Inclusion Bodies**

A characteristic, but by no means universal morphological change in virus-infected cells is the formation of inclusion bodies which are recog-
PLATE 6-1. Types of viral inclusion bodies (H and E stain, ×200). (A) Intranuclear inclusions; cells form syncytium—herpesvirus. Small arrow, nucleolus; large arrow, inclusion body. Note also margination of chromatin. (B) Intracytoplasmic inclusions—reovirus. Arrows indicate inclusion bodies in perinuclear locations. (C) Intranuclear and intracytoplasmic inclusions; cells form syncytium—measles virus (also seen with distemper and rinderpest viruses). Small arrow, intracytoplasmic inclusion body; large arrow, intranuclear inclusion body. (Courtesy I. Jack.)

FIG. 6-1. Inclusion bodies in virus-infected cells. (A) Vaccinia virus—intracytoplasmic acidophilic inclusion. (B) Herpesvirus—intranuclear acidophilic inclusion; cell fusion produces syncytium. (C) Reovirus—perinuclear intracytoplasmic acidophilic inclusion. (D) Adenovirus—intranuclear basophilic inclusion. (E) Rabies virus—intracytoplasmic acidophilic inclusions (Negri bodies). (F) Morbillivirus—intranuclear and intracytoplasmic acidophilic inclusions; cell fusion produces syncytium.
nized with the light microscope by their staining behavior (Plate 6-1). Depending on the virus, such inclusions may be single or multiple, large or small, round or irregular in shape, intranuclear or intracytoplasmic, and acidophilic or basophilic.

The most striking viral inclusion bodies are the intracytoplasmic inclusions found in cells infected with poxviruses, paramyxoviruses, reoviruses, and rabies virus, and the intranuclear inclusion bodies produced by herpesviruses, adenoviruses, and parvoviruses (Fig. 6-1). Some viruses, e.g., canine distemper and rinderpest viruses, may produce both nuclear and cytoplasmic inclusion bodies in the same cell. Many such inclusions have now been shown, by fluorescent-antibody staining or electron microscopy, to be accumulations of viral structural components, e.g., the nucleocapsids of paramyxoviruses. The basophilic intracytoplasmic inclusions invariably found in cells infected with poxviruses are the sites of viral synthesis (viral "factories"). Other very prominent inclusion bodies found in the cytoplasm of cells infected with fowlpox, ectromelia, and cowpox viruses, are acidophilic; these represent accumulations of viral protein and may or may not contain numerous mature virions.

In a few instances (e.g., adenoviruses, reoviruses) inclusion bodies represent crystalline aggregates of virions. Other inclusion bodies, such as those found in the nucleus of cells infected with herpesviruses, are the result of late degenerative changes which produce margination of chromatin.

**Cell Fusion**

A conspicuous feature of the infection of cell monolayers by paramyxoviruses, herpesviruses, and some coronaviruses and poxviruses is the production of syncytia, also called polykaryocytes or giant cells (see Plate 27-2). Late in their replication cycle, these viruses cause changes in the cell membrane which result in the fusion of the infected cell with neighboring uninfected cells. Such syncytia are often seen in the tissues of animals infected with these viruses.

If present at high multiplicity, paramyxoviruses can also cause rapid fusion of cultured cells to form syncytia. Cell biologists have used this phenomenon to produce functional heterokaryons by fusing different types of cells; for example UV-inactivated parainfluenza virus was used to produce "hybridoma cells" by fusion of antibody-producing B lymphocytes with myeloma cells in the pioneering experiments that produced the first monoclonal antibodies.
Nonspecific Histological Changes

In addition to changes due to the specific effects of viral replication, most virus-infected cells also show nonspecific changes, very much like those induced by physical or chemical agents. The most common early and potentially reversible change is what histopathologists call "cloudy swelling," which is associated with changes in the permeability of the plasma membrane. Electron microscopic study of such cells reveals diffuse swelling of the nucleus and distention of the endoplasmic reticulum and mitochondria. Later the nucleus becomes condensed and shrunken. Further cell destruction is the autolytic consequence of the leakage of lysosomal enzymes into the cytoplasm.

PERSISTENT INFECTIONS

Noncytocidal viruses, by definition, do not kill the cells in which they replicate. They often produce persistent infection, in which the infected cells produce and release virions but cellular metabolism is little affected and the infected cells continue to grow and divide. This type of cell–virus interaction is found in vertebrate cells infected with several kinds of RNA viruses: arenaviruses, retroviruses, and some paramyxoviruses, for example. In all these infections virions are released by budding from the plasma membrane of the cell. Although such virus-yielding cells may grow and divide in culture for long periods, there are slow, progressive changes that ultimately lead to cell death, except with retroviruses. In the animal, cell replacement occurs so rapidly in most organs and tissues that the slow fallout of cells due to persistent infection may have no effect on overall function. However, persistently infected differentiated cells may lose their capacity to carry out specialized functions (see below). Also, antigenic changes are produced in the cell membrane of persistently infected cells. In the animal, such changes may provoke an immunological response, which can rapidly lead to destruction of the cells (see Chapter 10).

The existence of persistent infection in cultured cells is demonstrable by a variety of laboratory procedures, such as hybridization with nucleic acid probes, staining with fluorescent antibody, hemadsorption, interference with the replication of a superinfecting virus, or electron microscopy.
EFFECTS OF NONCYTOCIDAL VIRUSES ON FUNCTIONS OF SPECIALIZED CELLS

Although they do not immediately kill cells, infections with non-cytocidal viruses often interfere with the specialized functions of cells. For example, murine neuroblastoma cells persistently infected with lymphocytic choriomeningitis virus fail to produce acetylcholine, although functions concerned with cell survival are unaffected. Viruses may also interfere with the secretion of immunoglobulins by lymphocytes and hormones by somatotrophic cells, e.g., the β cells of the islets of Langerhans, without killing the cells concerned. It is likely that these subtle effects, which can only be detected with special techniques, are quite common. Clearly, they may be of considerable importance in the infected animal.

Studies of respiratory epithelium in explant cultures have shown a specific functional response mimicking in vivo infection; rhinovirus infection results in cilial stasis and later in the destruction of cilia, although the cells are often not killed (Plate 6-2). This pathophysiological effect is important in the animal, because it lowers the resistance of the respiratory tract to secondary bacterial infection.

PLATE 6-2. The direct cytopathic effect of rhinovirus on bovine tracheal epithelium, as shown by scanning electron microscopy of explant cultures. (A) Normal appearance of ciliated cells. (B) Six days after infection many cells are rounded up or detached. [From S. E. Reed and A. Boyde, Infect. Immun. 6, 68 (1972).]
NEW ANTIGENS IN THE PLASMA MEMBRANE OF INFECTED CELLS

In many viral infections new virus-specified proteins are inserted into the plasma membrane of infected cells. For example, the plasma membrane of cells infected with enveloped RNA viruses (e.g., influenza virus, paramyxoviruses, and togaviruses) incorporates viral hemagglutinin, demonstrable by adsorbing red blood cells to their surface (hemadsorption). New virus-specified proteins appear in the plasma membrane quite early in the course of infection with many viruses.

Viral glycoproteins are not inserted at random in the plasma membrane. Cells in culture can be shown to be polar; their basolateral surface, in contact with the solid substrate, is different from their apical surface, which is in contact with the medium. In cultured canine kidney cells, influenza and parainfluenza viruses mature by budding from the free apical surface, but vesicular stomatitis virus (a rhabdovirus) and some retroviruses bud from the basolateral membrane. In each case the site of maturation is determined by the site of insertion of the viral glycoprotein into the plasma membrane, which is in turn a function specified by its nonglycosylated precursor. As discussed in Chapter 4, the precise nature of the "zip code" that determines the destination of any particular glycoprotein is unknown, but it may be of great functional significance in vivo. Viruses that mature at the apical surface of glandular epithelial cells are shed into the environment, whereas those maturing at the basolateral surface presumably move to other sites in the body, perhaps entering the bloodstream and establishing systemic infection. An example of this phenomenon in vivo is the polarized budding of rabies virus in the salivary gland, from the apical end of mucous epithelial cells into the salivary duct (see Plate 10-3).

Virus-coded antigens in the plasma membrane constitute a target for the body's specific immune mechanisms, both humoral and cellular, which may destroy the cell before significant numbers of new virions are produced and slow down the progress of the infection, hastening recovery. In some cases the host immune response may precipitate immunopathological disease (see Chapter 10). Herpesvirus-infected cells acquire a capacity to bind immunoglobulin nonspecifically, because of the synthesis of herpesvirus-coded proteins with the properties of Fc receptors in the plasma membrane; this, in turn, may lead to the destruction of the cell by antibody-dependent cellular cytotoxicity. Another class of new antigens found on cell membranes are the transplantation antigens, which are found in cells that are transformed by viruses (see below).
**TABLE 6-2**  
*Viruses That Transform Cells in Vitro, and Induce Tumors in Vivo*

| Family        | Subfamily or genus | Transformation in vitro | Tumor induction in natural host | Viral genome | Production of virus |
|---------------|--------------------|------------------------|--------------------------------|--------------|-------------------|
| Papovaviridae | Papillomavirus      | +                      | Papilloma (rarely carcinoma)   | Episomal     | +(papilloma)       |
|               | Polyomavirus        | +                      | No, but carcinomas in newborn rodents | Integrated  | -(carcinoma)      |
| Adenoviridae  | Mastadenovirus      | +                      | No, but carcinomas in newborn rodents | Integrated  | –                 |
|               | Aviadenovirus       |                        |                                |              |                   |
| Herpesviridae | Gammaherpesvirinae  | +                      | Lymphoma, carcinoma            | Episomal     | –                 |
| Hepadnavirida | Hepadnavirus        | –                      | Carcinoma                      | Integrated   | –                 |
| Retroviridae  | Oncovirinae         | +                      | Leukemia, sarcoma              | Integrated   | +                 |

*Some members only, in most groups.

bSee Chapter 12 and relevant chapters of Part II for further details.
TABLE 6-3
Characteristics of Cells Transformed in Vitro by Viruses

1. Greater growth potential in vitro
   (a) formation of three-dimensional colonies of randomly oriented cells in monolayer culture, usually due to loss of contact inhibition
   (b) Capacity to divide indefinitely in serial culture
   (c) Higher efficiency of cloning
   (d) Capacity to grow in suspension or in semisolid agar (anchorage independence)
   (e) Reduced serum requirement for growth
2. Altered cell morphology
3. Altered cell metabolism and membrane changes
4. Chromosomal abnormalities
5. Virus-specified antigens and DNA
   (a) New surface antigens (transplantation antigens)
   (b) New intracellular antigens (e.g., T antigens)
   (c) Some viral DNA sequences present, integrated with cellular DNA or as episomes
6. Capacity to produce malignant neoplasms when inoculated into isologous or severely immunosuppressed animals

CELL TRANSFORMATION

Viruses of several families can greatly change the growth characteristics of cultured cells, the process being called cell transformation. This phenomenon is correlated with the ability of the virus to induce tumors in animals (Table 6-2). Transformation by DNA viruses is always nonproductive (i.e., the transformed cells do not yield infectious progeny virus); transformation by retroviruses, on the other hand, is usually productive. Viral (or proviral) DNA in transformed cells is integrated into the cell DNA, except in the case of papillomavirus and herpesvirus DNAs, which remain episomal.

Transformed cells differ in many ways from normal cells (Table 6-3). One of the changes is an increased mitotic rate; transformed cells acquire a capacity to divide unrestrainedly, which can be demonstrated in a variety of ways (Plate 6-3), including the capacity to produce tumors in "nude" mice (which have defective cellular immunity, but do not support the growth of normal foreign cells).

Virus-Specific Antigens in Transformed Cells

Cells transformed by nondefective retroviruses express the full range of viral proteins, and new virions bud from their plasma membranes. In contrast, transformation by DNA viruses occurs only in cells undergoing
nonproductive infection; nevertheless, certain virus-specific antigens are regularly demonstrable. Tumor-associated transplantation antigens are located in the plasma membrane, whereas the so-called tumor (T) antigens are usually found in the nucleus. The proteins that are collectively called the T antigens play an important role in transformation (see Chapter 12).

INTERFERENCE AND INTERFERONS

Viral interference is said to occur when a cell infected by one virus resists superinfection with the same or a different virus. The interfering virus does not necessarily have to replicate to induce interference, and the ability of the challenge virus to replicate may be completely or only partially inhibited. Two main mechanisms have been clearly demonstrated: (1) interference mediated by defective interfering mutants and operating only against the homologous virus (see Chapter 5), and (2) interference mediated by interferon.
The last category is the most important and has generated a vast amount of research since its discovery by Isaacs and Lindenmann in 1957. As this research was stimulated primarily by the possibilities of treating human viral infections and human tumors, much of the work has been done with human interferons, now produced by genetic engineering, but the principles learned are of general application. The importance of interferons in veterinary medicine is currently limited to their role in recovery from viral infections (see Chapter 8); studies are in progress to determine whether and how they might be used for treatment of animal diseases.

Interferons

Most virus-infected cells produce a set of proteins called interferons, which are released and react with uninfected cells so as to render them resistant to infection with viruses. Some 17 human interferons have been identified and characterized, and there are probably a comparable number in other animal species. They fall into three antigenically and chemically distinct types, known as α, β, and γ (Table 6-4). All mammalian species have complex families of genes encoding different subtypes of interferon α, and one or two interferon β genes (except in cattle, which have multiple genes for interferon β). Nonmammalian verte-

| Property                  | Type | α   | β   | γ    |
|---------------------------|------|-----|-----|------|
| Produced by               |      |     |     |      |
| Leukocytes, epithelia     | α    |     |     |      |
| Viral infection or dsRNA  | β    |     |     |      |
| Lymphocytes               | γ    |     |     |      |
| Inducing agent            |      |     |     |      |
| Viral infection or dsRNA  | α    |     |     |      |
| Viral infection or dsRNA  | β    |     |     |      |
| Mitogens (nonsensitized lymphocytes) | γ |     |     |      |
| Number of subtypes        | 15   | 1   | 1   |
| Mr (major subtypes)       | 15,000–25,000 | 20,000 | 20,000–25,000 |
| Glycosylation             | No   | Yes | Yes |
| Stability at pH 2         | Yes  | Yes | No  |

aData from human interferons.

bMost subtypes, but not all.
brates have interferon β genes but no interferon α genes. Interferon γ is not induced by viral infection per se, but by lymphocytes following antigen-specific (or mitogenic) stimulation. It is really a lymphokine, which is regularly produced during the response to viruses in vivo (see Chapter 9), but not by virus-infected cell cultures. Its functions are chiefly immunoregulatory, although it does have some antiviral and antitumor activity.

Interferons α and β are not made constitutively in significant amounts, but are induced by most or all viruses when they replicate, in virtually all vertebrate species. Most RNA viruses are good interferon inducers; most DNA viruses, except for poxviruses, are rather poor. Some interferons, especially β and γ, display a certain degree of host species specificity; e.g., rabbit interferons are ineffective in mice or humans. However, there is no viral specificity, e.g., interferons induced by a paramyxovirus are effective against a togavirus or any other sensitive virus. Nevertheless, it is likely that individual subtypes of interferon, purified following cloning by recombinant DNA technology, will be found to be more effective against some viruses than against others.

**Biological Actions of Interferons.** Interferon was discovered as an antiviral agent, defined accordingly, and generally regarded as such by virologists for two decades. It is now widely acknowledged that the interferons probably represent a family of "nonclassical hormones" which lead, under defined circumstances, not only to inhibition of viral replication, but also to modulation of the immune system and several additional phenomena.

The mechanism of antiviral action of interferon α and β has been elucidated, in part at least. Interferon binds to a specific receptor on the plasma membrane, thus triggering a cascade of biochemical events (Fig. 6-2). Three new enzymes are induced: (2'-5') (A)n synthetase (often abbreviated to 2-5A synthetase), RNase L (endoribonuclease), and a protein kinase. By different mechanisms these enzymes inhibit protein synthesis in interferon-treated virus-infected cells.

It is also clear that interferons may stimulate or inhibit various arms of the immune response. One can think of interferons as lymphokines, induced principally by viral infection, and playing a key role in the regulation of the immunological response to that virus (see Chapter 9). Interferons secreted by virus-infected target cells, as well as by lymphocytes, macrophages, and NK cells, activate Tc lymphocytes, macrophages, and NK cells in the immediate vicinity to develop their cytotoxic potential, while also enhancing the expression of both class I and class II MHC antigens on the surface of the cells with which such
leukocytes interact. In turn, these effector cells not only destroy the target but produce more interferon following contact with viral antigen on the cell surface. The consequential cascade greatly amplifies the lytic arm of the immune response. Yet it is apparent that other important arms of the immune response are depressed by interferon, perhaps reflecting the propensity of interferon to inhibit the replication of lymphocytes. A full analysis of this complicated issue must await careful in vitro studies of the various actions of purified preparations of each cloned subtype of interferon on cloned populations of well-characterized lymphocyte subtypes. Armed with that knowledge, it should then be feasible to go back to the whole animal to document the biological relevance of each of the diverse effects of various interferons.

Fig. 6-2. Antiviral action of interferon (IFN): postulated pathways (see text). (From D. O. White, "Antiviral Chemotherapy, Interferons and Vaccines." Karger, Basel, 1984.)
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