VIRAL DISEASES OF THE NEXT CENTURY

MICHAEL B. A. OLDSTONE* and JUAN CARLOS DE LA TORRE

Since their discovery nearly one hundred years ago (1–3), viruses have been known by the diseases they cause. These diseases are characterized by three common events. First, the clinical presentation; second, the geography of the histologic injury; and third, the accompanying inflammatory infiltration (4). This is the portrait of acute virus infections. Yet, it is becoming more evident that viruses also cause persistent infections. Viruses that persist in a host are ultimately successful according to their abilities 1) to survive within cells that provide their sustenance and 2) to avoid recognition by the host's immune system. Thus, the virus that persists must evolve strategies of how to live symbiotically within a host over the latter's lifetime. A successful plan necessitates the absence of cell lysis and inflammation, the cardinal signs associated with acute viral infections. In the past and currently virologists and infectious disease specialists have focused attention on agents that have failed to solve this puzzle because they display destructive (lytic) behavior, frequently causing disease. This scenario makes them easy to detect. As a result of such short-sighted abuse of the host, these viruses need to continually seek new cells and/or hosts to infect. It is the study of such viruses that has dominated the first century of virology.

Viruses that cause acute tissue destruction, resulting in significant morbidity and mortality, do so by disrupting membranes or shutting down host protein synthesis. Because such viruses multiply exponentially, and host protein synthesis is shut off, viral markers are easily recognized by using biochemical or molecular assays. In addition, these viruses trigger an attack on the cells they infect by killer cytotoxic T lymphocytes (CTL) of the immune system. The scenario is as follows: killer CTL, stimulated and numerically expanded by antigens of the virus, recognize viral fragments displayed on the infected cell surface by a protein of the class I major histocompatibility complex.

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*Head, Viral-Immunobiology Laboratory and Division of Virology, Department of Neuropharmacology, The Scripps Research Institute, La Jolla, California.

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Address to which requests for reprints should be sent: Department of Neuropharmacology (IMM-6), The Scripps Research Institute, 10666 N. Torrey Pines Road, La Jolla, CA 92037.

This process entails antigen presentation, MHC restriction, CTL recognition and activation, and the release of cytotoxic proteins or enzymes from CTL that destroy the infected target (reviewed in 5). Host defenses, such as CTL, have evolved to limit the production and spread of lytic viruses by removing the cellular factories that enable them to reproduce. Viruses that persist must, first, remain within a cell for a prolonged time without disturbing the transcription or translation of genes necessary for the infected cell's survival or altering lysozomal or plasma membranes or cytoskeletal structures. Second, such viruses must interfere with antigen presentation, MHC restriction, CTL activation and/or CTL activity. Table 1 lists the viral strategies of persistence. Highly differentiated cells within the host that do not have the potential for regeneration, i.e., neurons, have evolved special strategies to prevent antigen presentation and MHC restriction of viral peptides on their plasma membrane surfaces, thus rendering them invisible to meandering killer T cells. Indeed, recent data from our laboratory (6, 7) document such strategies, in that virally infected neurons have a defect in transcription of the α chain of MHC (6) and of molecules that translocate peptides from the cytosol to the ER compartment (7) where MHC molecules are synthesized.

For viruses to persist, they must 1) evolve strategies to avoid immune surveillance and 2) develop pathways of nonlytic replication in the cells they infect. Mechanism by which this occurs is outlined in Table 1.

Unless the host-virus relationship is totally symbiotic for both partners, the host cell pays a price to ensure its survival during viral persistence. It has become clear over the last decade that certain persistent viruses can interfere subtly with cells' ability to produce differentiated products (hormones, neural transmitters, cytokines and

TABLE 1
Principles of Viral Persistence

Avoid Immunologic Surveillance
1. Remove recognition molecules on infected cells
Alter expression of viral protein
Alter expression of MHC molecules
Alter expression of adhesion molecules
2. Abrogate lymphocyte/macrophage function
Immunosuppression
3. Hide in cells lacking MHC expression (neurons)
4. Generate antibody and CTL escape variants
Non Lytic Phase of Viral Replication
1. Generate mutants or variants
2. Diminish expression of viral genes or their products

immunoglobulins) without disrupting the cells' vital functions (respiratory enzymes, cytoskeletal proteins, lysozomal and plasma membrane integrity, etc.) (reviewed in 8). By this means, the virus can replicate in cells that appear histologically normal by light or high resolution electron microscopy, although the function of the cell is altered. Figure 1 presents a cartoon of this effect. Further, since virally infected cells have evolved strategies to escape immunological surveillance, the ordinarily expected T lymphocyte and monocyte infiltration in their immediate neighborhoods does not occur. Despite viral replication, such infected cells maintain their normal anatomical architecture, yet the virus disorders the differentiated or luxury function of a cell, often leading to disturbances in homeostasis, and eventually disease.

Realizing that viruses persist and thereby cause disease has been one of the major accomplishments in virology. The principles by which viruses persist, and how such persistent infection leads to disease has

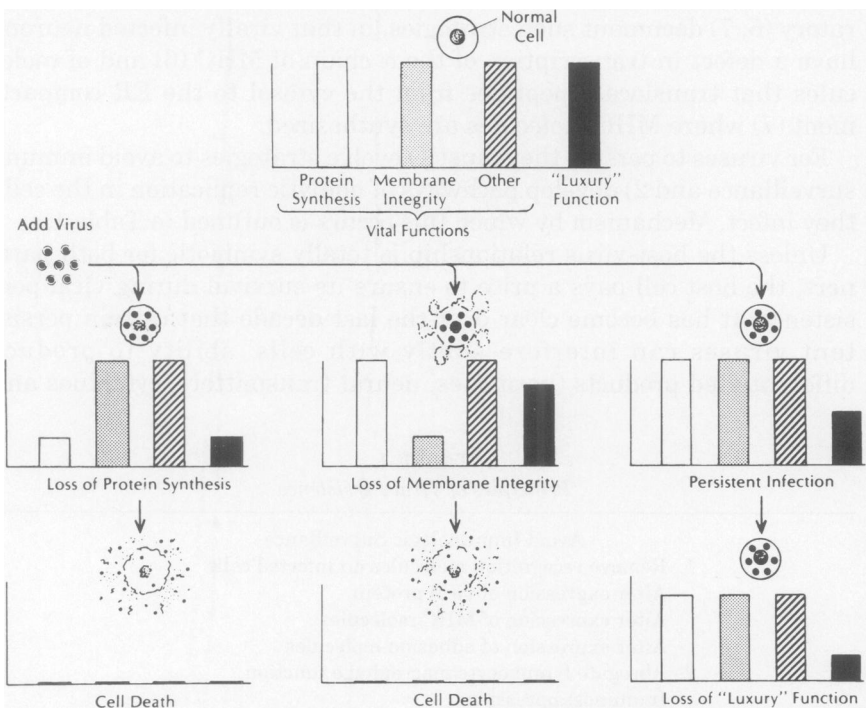

FIG. 1. Cartoon of two ways viruses can cause cell dysfunction, injury and disease. In the first, viruses alter the vital function of cells and cause their eventual lysis by shutting off protein synthesis or by interfering with the integrity of membranes. By the second pathway, viruses do not lyse a cell or shut down protein synthesis, but do interfere with the cell's ability to make such products as a hormone, immunoglobulin, or neurotransmitter. This lecture focuses on the second path of viral action.

been the long-term interest of our research group. Our focus has been on the ability of certain viruses to interfere subtly with cells' ability to produce differentiated products such as hormones, neural transmitters, cytokines, and immunoglobulins, etc., in the absence of a virus-induced cytolysis or inflammation (reviewed in 8).

The evidence that led to this concept came initially from our studies of neuroblastoma cells that were persistently infected with a nonlytic RNA negative strand virus, lymphocytic choriomeningitis (LCMV) (9, 10). These cells make the enzyme choline acetyltransferase, which is needed to synthesize acetylcholine, as well as the enzyme acetylcholine esterase that degrades acetylcholine. When persistently infected, more than 98% of such cells expressed LCMV antigens for several months and had growth rates and cloning efficiencies equivalent to uninfected neuroblastoma cells cultured in parallel. Yet, these persistently infected cells displayed a significant decrease in both the transferase and esterase enzymes required for the synthesis and degradation of acetylcholine. Thus, these observations showed that a persistent viral infection failed to alter the histopathologic or anatomical profile of the cells it infected, and failed to alter the vital functions of the cells, but nevertheless significantly altered the differentiated or luxury function of these infected cells.

Similar observations by others (11, 12) showed a similar effect in chick chondroblasts, myeloblasts, or melanoblasts infected with a temperature-sensitive mutant of Rous sarcoma virus. At temperatures that were non-permissive for viral replication, the virus had no effect on the chondroblasts' ability to make sulfate-proteoglycans, on the muscle cells' production of myotubules or heavy and light chains of myosin, or on melanoblasts' manufacture of melanin granules. However, when temperatures were shifted to allow viral replication, chondroblasts failed to make sulfate-proteoglycans, muscle cells failed to make myotubules or heavy and light chain of myosins, and melanoblasts were deficient in production of melanin granules. Soon after, many researchers reported similar observations of a wide range of RNA and DNA viruses infecting a diffuse collection of cells, including those of neural, glial, lymphocyte, macrophage, fat, and muscle origin (reviewed in 13). In all such cases, replicating viruses did not lyse the cells they infected, but, instead, disturbed the expected functions of such cells.

It was important to know whether these *in vitro* observations also occurred *in vivo* and caused disease. We showed that LCMV, replicating in growth hormone (GH)-producing cells of the anterior lobe of the pituitary of infected C3H/ST mice, caused a deficiency of GH synthesis without disturbing the cells' architecture or lysing them (14–17). Viral

infection of such cells was not accompanied by an infiltration of inflammatory cells. However, the deficiency in GH synthesis interfered with the animals' growth and development, and lowered carbohydrate metabolism that continued to fall until most mice died by 30 days after initiating viral infection. Adoptive transfer of GH-synthesizing cells into such mice corrected the glucose levels, permitted survival, and restored normal growth, as judged by increases in body weight and length (15). Biochemical analysis of this GH deficiency indicated that the defect lay at the initiation of transcription of the GH gene (18, 19).

To unravel the molecular mechanisms involved, we utilized a cultured pituitary cell (PC) line. Individual PC cells express both GH and prolactin (PL) and with LCMV infection, more than 98% of cells express viral progeny. Their growth rates and cytomorphology duplicate those of uninfected cells but infected cells could not transcribe GH mRNA, although their transcription of PL was normal or only slightly altered (20). Detailed molecular studies of such infected PC indicated that the virus affects the GH promotor by its activity on the transactivator of GH, GHF1 (PIT-1) (20).

Subsequently utilizing animal models it became clear that the concept of nonlytic virus(es) disordering homeostasis and causing disease was a general phenomena. In addition to the growth retardation and hypoglycemia discussed above, several DNA and RNA viruses could alter the function of the thyroid gland, beta cells in the islets of Langerhans, neurons, and cytotoxic T lymphocytes, disordering these cells' differentiated functions in the absence of cell lysis and causing endocrine, neurologic or immune diseases (reviewed in 8, 13).

It is likely that we are bathed in a sea of microbes, yet are harmed by relatively few. Viruses are probably responsible for a wide variety of clinical illnesses whose cause is currently unknown. By adopting a symbiotic type of relationship with the cells they infect, and through alteration of these cells' differentiated functions, viruses, in the course of persistent infections, affect numerous biological systems. Because viruses disorder the function of cells without killing them, there is an opportunity to reverse the diseases they cause by the use of antiviral therapy (20–23). Ribavarin, an anti-viral drug, is effective against several arenaviruses including LCMV. Treatment of LCMV-infected PC not only clears the virus infection, but restores the synthesis of GH *in vitro* (20). Studies utilizing ribavarin and its analogs in vivo in GH-deficient mice are currently in progress. Experimental results in multiple laboratories have documented that persistent LCMV infection is associated with abolition of the CD8 virus-specific MHC-restricted CTL response (reviewed in 5). Adoptive transfer of such cells into virally in-

fected mice clears the persistent infection (21–23) from cells in the body including neurons, lymphocytes and macrophages in blood or spleen and in many instances restores a disordered homeostasis to normal.

As we learn how to culture differentiated cells like neurons, oligodendrocytes, beta cells of the islets of Langerhans, etc., we are certain to uncover persisting viruses and likely to find more diseases currently of unknown etiology, to be caused in humans by virally induced alterations of cells' functions without lysis of those cells. The potential grows then for using antiviral therapies to restore normalcy and correct such diseases.

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DISCUSSION

President Allen: Michael, I wonder have you, in your hypotheses, considered the possibility that in addition to causing disease, viruses intrinsic to cells are part of the normal differentiation process and, in fact, become symbiotic with human life?

Dr. Oldstone: Yes, but the issue that I am discussing today is a host-virus relationship that is not a perfect marriage. The virus is still a little macho because it causes some injury. The question is, can viruses act symbiotically to, in fact, potentiate good things in host cells? There is some evidence that viruses may do good things. Some viruses, in fact, encode a variety of genes that are important for epithelial growth. They can also encode some interleukins and cytokine genes. Of course, they may carry such genes to better allow their own survival and for their own benefit. There is no reason why viruses can't be looked upon, (you realize that La Jolla is only about 50 miles south of Disneyland, so you will have to allow me some fantasies), as bacteria are in terms of being able to prove beneficial products for mankind, in terms of some of the genes that they carry.

Dr. Robert Flynn, Wilmington: An interesting, wonderful presentation. I am tempted, however, to ask the question: If a virus can decrease function of a cell, why does it have to be reversible? Could it not selectively eventually damage that function beyond the point of repair but not kill the cell?

Dr. Oldstone: Yes, it is a matter of degree. You are perfectly right, viruses could do that, but we are talking about viruses that evolved a strategy to live symbiotically or near symbiotically within a cell over the lifetime of the cell it infects. It turns out that the manufacture of growth hormone by growth hormone-producing cell is probably not essential for that cell's survival. It may be essential for the survival of the host, but certainly not essential for that particular cell. The virus doesn't turn off the respiratory enzymes or the vital enzymes that are necessary to keep the cell alive, but it turns off these luxury products. Why the virus I presented selectively interferes with growth hormone is unknown but may provide some selective advantage to the virus.