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Seminars in Virology

A Biological Perspective of Slow Virus Infection and Chronic Disease

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Sequential events characterize the interaction of viruses with parenchymal cells, and acute lytic infections of tissues and organs have broad biological attributes. A knowledge of these permits a keener understanding of persistent, intermittent *herpesvirus infections and* persistent, continuous *respiratory virus infections. In addition to unique biochemical mechanisms which may permit the latter chronic infections to evolve, the roles of defective and mutant strains of virus, viral interference, and the genetic, developmental and immunological expressions of the host are of considerable and provocative importance.*

The traditional view of viral infections embraces a broad spectrum of acute pathological and inflammatory events. The relationship of measles virus to subacute sclerosing panencephalitis, the elucidation of the latency of herpes simplex virus, and the slow unmasking of the pathogenesis of multiple sclerosis have illustrated the subtle elements of persistent viral infections of the human being. These chronic neurological diseases have provided the opportunity and stimulus for sharp dissection of the biological and biochemical processes which embellish the logical link of viral infections to other forms of chronic human illness.

ONE OF THE most exciting periods of modern virology has evolved during the past several years. Suspected for a long time, the pathogenic relationship of human viruses to chronic illness is beginning to assume plausible and provocative attributes. The mystery of viral persistence, altered cellular physiology and immunological control mechanisms is being solved by careful biological research and extensive, multidisciplinary epidemiological studies.

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Host Cell-Virus Interaction and Cytoanatomical Events

It is important to know how virus particles interact with a mammalian cell. Mature particles attach to receptors on the cell surface where the early phases of uncoating of the viral capsid (protein coat) and penetration ensue. For many of the human viruses which are suspected to be causes of chronic diseases, the surface of the virion and the surface of the cell fuse and only

ABBREVIATIONS USED IN TEXT

CMV = cytomegalovirus
DNA = deoxyribonucleic acid
EBV = Epstein-Barr virus
HSV = herpes simplex virus
RNA = ribonucleic acid
SSPE = subacute sclerosing panencephalitis
T-cells = thymus-derived lymphocytes
ts = temperature-sensitive
VSV = vesicular stomatitis virus
VZV = varicella-zoster virus

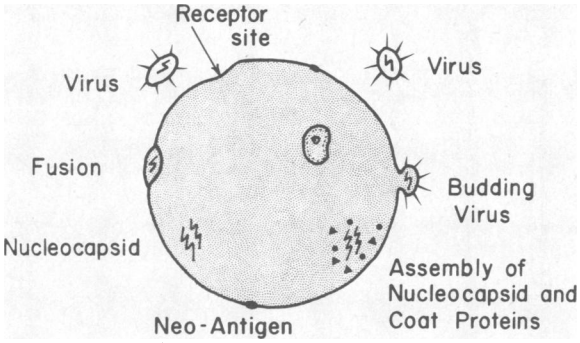

Figure 1.—Interaction between virus and host cell, including: the early phases of exposure, attachment, fusion-penetration and eclipse; the subsequent synthesis of new viral cytoplasmic components and cell surface antigens; and the final enveloping, budding and release of progeny virions.

the genetic material of the virion, the nucleocapsid, actually enters the cell. Implemented by viral and cellular enzymes, the genetic material of the virus is transcribed and translated into new genome, enzymes and structural proteins sufficient for the assembly of progeny virus particles. These subtle and complicated biochemical events transpire quickly during a period of eclipse when viral particles or subunits can no longer be detected by ultrastructural analysis. As new viral components are synthesized, they aggregate beneath the plasma membrane of the host cell, become packaged into new virions, are enveloped by host membranes and, finally, are budded off the surface of the cell into the extracellular environment (see Figure 1). In the course of these virus-induced intracellular events, the plasma membrane may be altered slightly by the substitution of glycoprotein units of the viral coat as new antigenic determinants on the cell surface.

The Biology of Acute Viral Infection

Extending the background of understanding, it is important to note the impact of an acute, lytic viral infection upon the host cell. The diversion

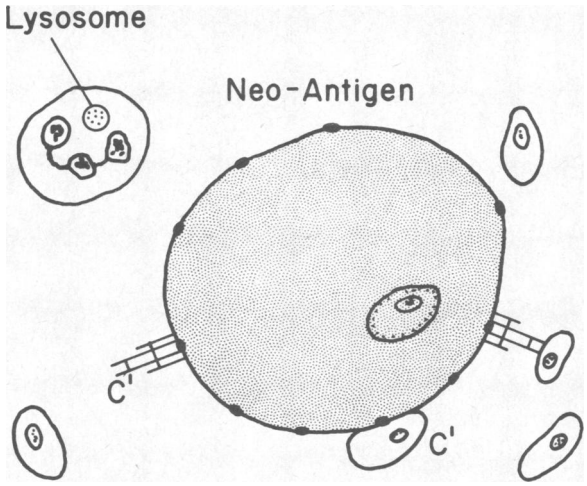

Figure 2.—Immunological and inflammatory recognition of an infected cell with viral-programmed surface antigenic determinants and the immunocytopatholytic events mediated by T-lymphocytes, monocytes, antibody-dependent killer mononuclear cells and the interaction of complement (C′) with specific antibody and immunocytes. Polymorphonuclear leukocytes, containing potent lysosomal enzymes and phagocytic function, execute a final inflammatory dissolution of damaged cell populations.

of biochemical processes to the production of new viral particles may be accompanied by a shutting-off of intrinsic cellular deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and protein synthesis. The appearance of new antigenic determinants on the cell surface permits immunological recognition by specific viral antibody, thymus-derived lymphocytes (T-cells) and complement (see Figure 2). Their aggregation about the infected cell produces immunopathic cytolysis and the elimination of damaged, virus-releasing cells. Polymorphonuclear leukocytes and mononuclear macrophages also participate in this inflammatory pathologic process. These cells are attracted to the scene by chemotactic immunoproteins and complement, and contribute to the final dissolution of host cells bearing persistent virions, viral genome or nucleocapsid structures.

These processes explain the characteristic histopathology and pathophysiology of acute viral infections, including: (1) interstitial pneumonia, surfactant-depleted atelectasis, ventilation-perfusion dysequilibrium and hypoxia; (2) intraluminal gastrointestinal solute and water loss and transient malabsorption of fat and carbohydrate; (3) vasculopathic cerebral edema, meningeal inflammation, cortical and cord neuronal dysfunction and paresis; (4) hepatocytic necrosis and edematous canalicular obstruction, and (5) the

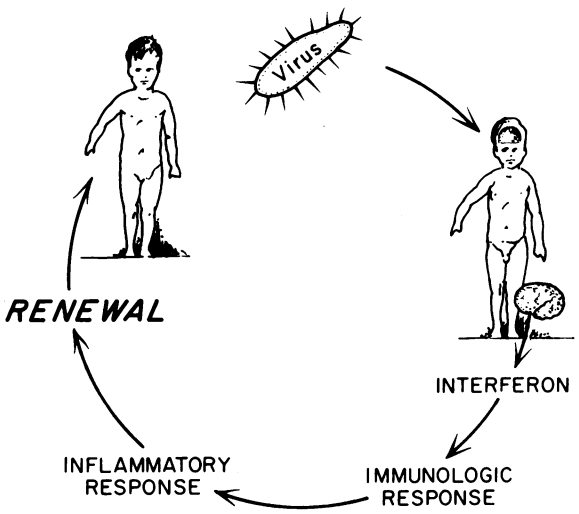

Figure 3.—Sequence of acute viral infection; altered histopathology and pathophysiology; the elaboration of regulatory antiviral protein, cellular and humoral immunity, and inflammation; and the ultimate restoration of normal structure and function.

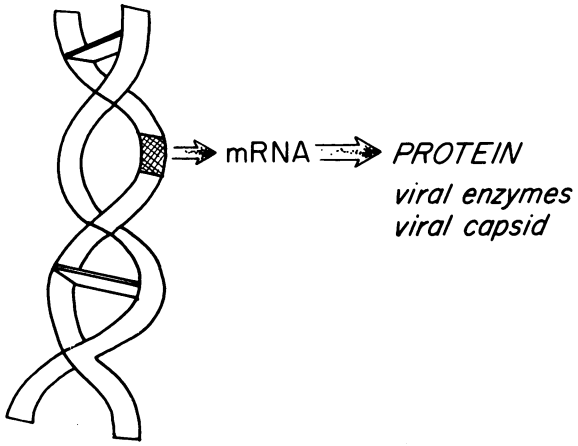

Figure 4.—Persistent, intermittent infection with the integration of viral DNA (hatched) into host cell DNA and the subsequent transcription of messenger RNA (mRNA) for the synthesis of essential enzymes and coat (capsid) proteins for new virions.

epidermal necrosis and perivascular inflammation which characterize the vesicular and morbilliform exanthems.

With acute viral infection there exists ample opportunity for restoration of damaged and destroyed cells within an organ. As antibody production and T-cell mediated cellular immunity unfold and are embellished by inflammation and the capability of host cells to synthesize interferon, an important regulator of subsequent viral replication, the burden of virus subsides and cellular renewal begins (see Figure 3). In most instances, patients are left without any anatomical or physiological residue.

Persistent, Intermittent Viral Infection

The herpesviruses provide a classic example of chronic viral infection. This family of viruses, herpes simplex virus (HSV), varicella-zoster virus (VZV), cytomegalovirus (CMV) and Epstein-Barr virus (EBV), are apparently able to position their viral DNA either into the DNA of the host cell or very close to it. This "integration" is important because all of these viruses produce relapsing, reactivation forms of illness, possibly even EBV.

The clearest understanding of this relationship involves HSV. HSV DNA may be "integrated" as latent genetic material in the nucleus of ganglionic neurons (see Figure 4). The experiments of Stevens and his colleagues[1-3] have elucidated this exciting biology in an elegant fashion. These workers have suggested that specific HSV neutralizing antibody may hold the genome in check,[4] although one suspects that cell-mediated immunity is a more critical determinant of control of HSV reactivation. When one of the many events which permit derepression of this viral genome occurs, the full-scale production of mature HSV virions is triggered within the neuron and new viral particles flow down the axoplasm to the epidermal surface where they evoke the lytic alterations of a recurrent, vesicular eruption. The same biological process is likely operative for the pathogenesis of herpes zoster or shingles. CMV and EBV may remain latent in a similar manner in epithelial and lymphoid cells which, in contrast to neurons, are capable of division.

DNA-viruses, including the adenoviruses,[5] seem to be uniquely capable of establishing latency followed by intermittent episodes of reactivation, repetitive viral shedding and acute clinical illness.

Persistent, Continuous Viral Infection

Following a primary overt or subclinical infection, virus may persist in the host cell for prolonged periods of time. Paramyxoviruses and arthropod-borne viruses (arboviruses) loom as the most likely candidates for establishing chronic infection in human tissues, particularly in cells which do not undergo constant renewal. A compelling example is the devastating disease subacute sclerosing panencephalitis (SSPE), in which measles virus nucleocapsid structures are found to cortical neurons and glial cells.[6] Although ultrastructural studies have not confirmed the presence of viral components, the vast source of

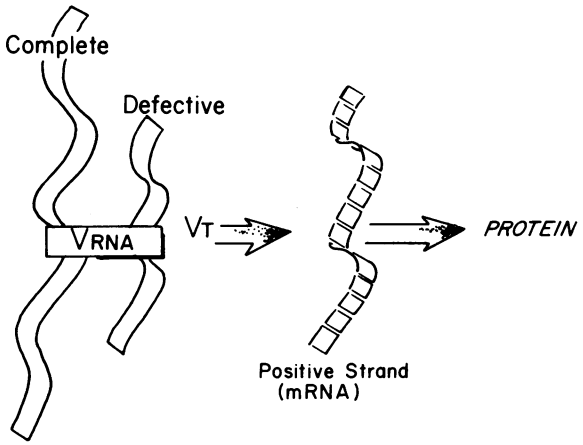

Figure 5.—Persistent, continuous infection with the presence of viral transcriptase (VT) enzyme which permits negative strand viral RNA (VRNA), the genome of myxoviruses and paramyxoviruses, to serve as the template for the RNA to RNA copying of positive strand viral messenger RNA (mRNA) and ultimate synthesis of viral proteins. Defective viruses compete with complete viruses for the critical use of VT and thus interfere with the replication process of complete viruses.

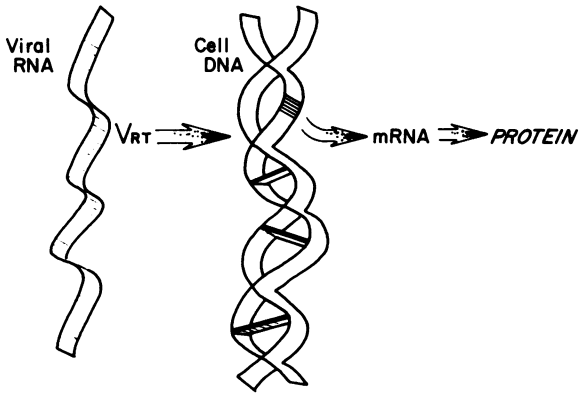

Figure 6.—Persistent, continuous infection with the presence of viral reverse transcriptase (VRT) enzyme which permits negative strand viral RNA to serve as the template for the reverse copying of an RNA to DNA segment which may be integrated into the DNA of the host cell.

serological and epidemiological data suggests that measles virus may be linked to multiple sclerosis, viral genome being reservoired in astrocytes and oligodendroglial cells which provide axonic myelin sheaths.

In contrast to the DNA-virus infections discussed above, the infections produced by these RNA-viruses may be constant, simmering processes.

Biochemical Aspects

Paramyxovirus RNA genome can be transcribed to produce messenger RNA molecules which code for new viral genome, enzymes and structural proteins. These events occur because of the inclusion of transcribing enzyme, RNA transcriptase, into the structural proteins of the mature virion (see Figure 5). It is conceivable that a virus so smartly prepared for its biochemical mission may produce new viral RNA within a cell which can persist undisturbed for many years. Persistent viral RNA may be capable of coding for special viral protein which, while failing to complete the process of viral replication, can alter either cellular function or surface membranes.

However, very recently a more plausible biochemical explanation for the persistence of paramyxoviruses has evolved. Similar to the biochemical function of mammalian and perhaps human RNA tumor viruses, several paramyxoviruses have been discovered to be capable of producing transfection[7] (see Figure 6). Transfection is a process by which viral RNA may transcribe or produce a DNA homologue which in turn may be approximated to the host cell DNA in anatomical location and, most important, in concurrent biological expression. The RNA tumor viruses possess a critically important enzyme as part of their constitutive structure which carries out this extraordinary biochemical annexation of fundamental host cell processes. The enzyme is aptly named reverse transcriptase, indicating the copying of DNA from RNA, in contrast to the RNA to RNA copying which is mediated by the aforementioned viral transcriptase. Indirect evidence suggests that under certain conditions respiratory syncytial virus, measles virus, rubella virus, Newcastle disease virus and visna virus possess reverse transcriptase enzyme.[7-9] Although respiratory syncytial virus and Newcastle disease virus have not been implicated in chronic disease processes, measles virus has been clearly linked to SSPE, possibly linked to multiple sclerosis, and evidence has been advanced recently to suggest that DNA copy of measles virus RNA is integrated into the host cell DNA of patients with systemic lupus erythematosus.[8] Furthermore, rubella virus has been isolated from the brain of a child with chronic panencephalitis and linked to chronic encephalopathy in other patients.[10]

Another explanation for the persistence of viral genome within the cell implicates a biochemical peculiarity of the host cell itself. Cellular protease enzymes cleave away some of the viral nucleocapsid, rigidifying nucleocapsid structure to such an extent that the nucleocapsid may not be able

to fold into the enveloping configuration of the viral protein coat and the lipids of the cellular plasma membrane.[11,12] The protease altered viral nucleocapsid may also lose ability to home into the budding site on the plasma membrane where the final assortment and packaging of viral components occur before completion of infection and release from the cells.

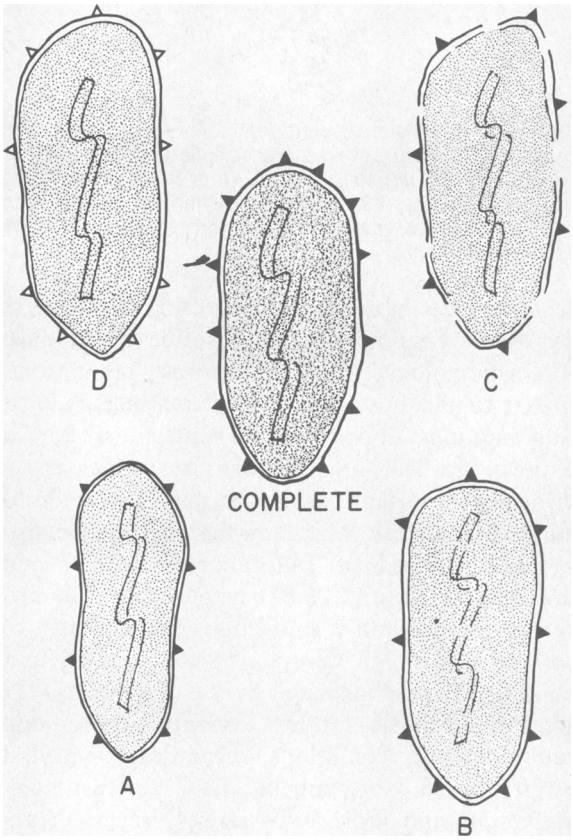

Figure 7.—Defective viruses may be smaller **(A)** than complete viruses, may contain genomic deletions **(B)**, may be encapsidated by unstable structural coat proteins **(C)**, or possess fewer external coat protein components **(D)**.

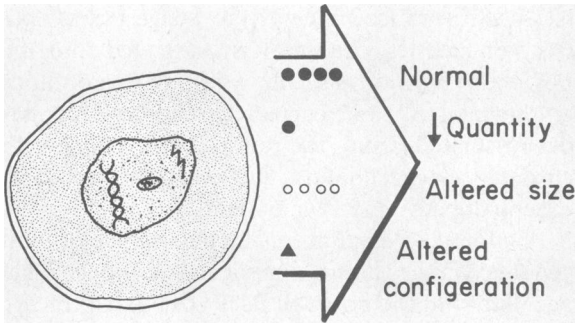

Figure 8.—Biochemical and physiological disturbance of the synthesis of protein product by a parenchymal cell with persistent, continuous infection.

Defective Viruses

In addition to these biochemical explanations for the persistence of viruses in human tissues, there exists the provocative biological phenomenon of defective viral particles and the expression of viral interference (see Figure 7). The quantitative anatomy of defective viruses is difficult to analyze. Precise biophysical evaluation of vesicular stomatitis virus (VSV), a virus of little human importance, indicates that defective VSV is smaller than complete mature VSV.[13] The same seems to be true for parainfluenza virus type 1 and possibly measles virus.[14,15] Other defective viruses, while of the same total size as standard complete virions, possess small deletions of their genome which impair their biochemical function during the early phases of viral replication. Defective Sindbis virus has a small lesion in the viral RNA which results in the transcription of smaller messenger RNA molecules with the ensuing production of fewer or unstable structural proteins for the viral coat.[16] Defective influenza virus has a decreased amount of transcriptase enzyme and synthesizes in the course of infection a decreased amount of the longer RNA segments which constitute the genome of virus.[17] Defective virions compete more successfully with standard virions for viral RNA polymerase enzymes and effectively turn off the reproduction of standard virus particles.[13,18] Defective viruses do not consummate their own replication very efficiently and they persist within cells without the provocation of the biochemical and immunological mechanisms which can destroy virus-producing cells. As an example, standard reovirus produces an acute encephalitis in mice, while defective reovirus produces a slowly evolving chronic hydrocephalus.[19] Perhaps, sufficient viral genome persists in the host cells to produce some regulatory proteins. If certain of these viral-coded proteins perturb the normal biochemical mechanism of parenchymal tissues, physiological malfunction of an organ might result. An example might be a diminished or abnormal protein product by a critical organ, such as insulin or thyroxin (see Figure 8).

Immunopathologic Aspects

One of the prevailing pathogenetic theorems for multiple sclerosis consists of direct viral involvement of oligodendroglial cells and the consequent impairment of myelin production.[20] A similar demyelinating process may occur if virus

disturbs the normal biological function of neurons with subsequent axon injury, followed ultimately and secondarily by demyelination. Extending the example of multiple sclerosis, immunological phenomenon may be overlaid on the initial biochemical disturbance within glial cells. If transfecting or defective viral nucleocapsids can persist within parenchymal cells, they may possess enough biosynthetic capability to alter the cell surface with new glycoprotein antigenic determinants. Low intensity immunoreactivity of the host may produce a more chronic autoimmune form of histopathology rather than acute immunopathic destruction of infected cells. This immunopathological process can escalate multiple sclerosis with inflammatory bursts and relapses. Immunopathic elements may also amplify the relentless course of chronic cerebral dysfunction in SSPE.[6] Thyroiditis, diabetes mellitus and the cardiomyopathies may represent illnesses where persistent forms of viral infection produce a primary direct biochemical and physiological hit on the host cell, followed by a secondary, chronic immunopathological inflammatory process. There is ample evidence for circulating and tissue-bound immunoproteins and T-cell lymphoid responses in both experimental and human thyroid and myocardial disease.[21] Although the relationship of group B coxsackie viruses to juvenile diabetes mellitus in humans remains equivocal, despite compelling evidence in experimental animals that enteroviruses can produce diabetes, the relationship of juvenile diabetes mellitus to mumps virus, another paramyxovirus, may prove to be real.[22-25]

Attenuated and Mutant Viruses

In closing discussion of the biochemical and biological attributes of persistent viruses, a comment should be made about attenuated viruses and temperature-sensitive (ts) mutants. Attenuated poliovirus, a ts mutant, consists of defective virions.[26] Mutant strains of measles virus, produced *in vitro* by chemical agents, can evoke modified infections in experimental animals, including a progressive form of hydrocephalus.[27] Ts mutants of vsv persist in the brains of mice, occasionally producing mild pathological changes in contrast to the fulminant disease of standard vsv particles.[28] Mutagenic chemicals have permitted the recovery of ts mutants of other paramyxoviruses, which offer for vaccine development the attractive aspect of replication only at the lower body temperature of the upper respiratory tract. Unfortunately, some of these ts mutants also possess genomic deletions which render these particles partially defective and potentially capable of persistence. To date, a relationship of attenuated human vaccine viruses with persistence and chronic disease has not been documented.

Ts mutants of parainfluenza virus type 1 are unable to direct the synthesis of a pivotal coat protein, the hemagglutinin-neuraminidase glycopeptide. While virus can replicate in host cells, the usual cytolysis does not occur, offering substantial concern about the possibility of persistence.[29] Although wild strains of Newcastle disease virus possess transcriptase enzyme, only mutant strains possess the reverse transcriptase so important for the phenomenon of transfection.[9] Measles virus ts mutants induce infection with the accumulation of viral antigen in the nucleus and cytoplasm of host cells but fail to code for effective coat protein function at the plasma membrane.[30]

The Host

The suggestion has already been made that the host cell may share some of the blame for chronic, persistent viral infections. Certain tissues and organs produce a greater population of defective particles than others and in the case of some experimental animal models this phenomenon is closely regulated by heredity.[31-33] Certain cells permit the expression of the phenomenon of viral interference more extensively.[34]

The immunological response of the intact host may be of critical importance in the pathogenesis of a chronic viral infection. Probing for a genetic linkage to susceptibility and immunological response, the haplotypes HLA-A_3, HLA-B_7, HLA-DW_2, and the new B group 4 have been found more frequently in patients with multiple sclerosis.[35] Although the data are inconsistent, multiple sclerosis patients may have diminished expression of cell-mediated immunity to measles virus and perhaps other viruses, despite the fact that T-lymphoid cells from these patients react more vigorously with measles virus-infected cells *in vitro*.[36-38] If cellular immunosuppression proves to be true and important, an explanation may be sought from the observation that measles virus replicates in mononuclear cells and could diminish the immunological function of these lymphoid cells.[39]

While it is logical to conclude that a specifically immunosuppressed host may encumber persistent viral infection, the permissive relationship of specific antibody to persistent infection is even more provocative. Constant low levels of humoral measles virus antibody can polarize or "cap" viral antigenic determinants on the cell surface and strip away the antigen, precluding the total dissolution of the virus-producing cell by immune complex or T-cell mediated immunocytolysis.[40] In this way, virus, possibly defective, maintains effective sanctuary. Interestingly, persistently infected cells produce much less plasma membrane measles virus antigen than cells infected with wild measles virus.[41]

Cells infected with defective virus are less able to envelop and release new virions, and so they are less susceptible to the removal of infected cells by immunocytolysis. More potent antibody to the most integral structural components of the viral capsid and envelope may be missing in individual humans or animals with defective mutant virus infection, simply because viral antigens are not delivered to the surface of the cell where the immunopoietic and immunocellular components of the host may recognize and respond.[42] In slow, persistent visna virus "transfection" of the sheep choroid plexus, only 0.1 percent of the cells containing reversely transcribed viral DNA produce complete virus and new viral proteins.[43]

It is important to realize, as has been suggested for the chronic, intermittent infections of the herpesviruses, that vigorous antibody response, and perhaps cellular immunity as well, may make the virus "take a dive" and become more intricately integrated into the structural and biochemical elements of the cell. In this regard, intracerebral infection of rhesus monkeys with human SSPE measles virus produces an acute, fatal, degenerative, relatively noninflammatory encephalopathy in nonimmune primates, whereas in naturally immune animals chronic, progressive encephalitis develops with elevated cerebrospinal fluid antibody, glial cell proliferation and perivascular inflammation.[44]

The age of the host is of considerable importance. Defective-interfering particles are elaborated more extensively by infant mice infected with vesicular stomatitis and rabies viruses.[45] Neonatal hamsters seem to be more susceptible to the chronicity of experimental measles virus infection than older animals.[46] There is intriguing epidemiological evidence from the Middle East suggesting that SSPE occurs more often in children in whom primary measles virus infection developed when they were infants rather than during later childhood.[47] Increased multiplication of measles virus has been noted in the mononuclear cells of newborn humans, presumed to result from the heightened DNA and RNA metabolism of infant cells.[39] Measles virus may gain access to more remote sites such as the central nervous system by hematogenous transport in leukocytes.

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Smoking, Alcohol and Cancer of the Larynx

LARYNGEAL CANCER seems to be related to consumption of alcohol. Even when we analyze the data from studies of laryngeal cancer and control for the amount of excess smoking that heavy drinkers engage in, we still see an increase in risk in cancer of the larynx due to alcohol consumption. It seems from recent studies, too, that the increases in risk for cancer of the larynx from tobacco smoke and from alcohol seem to be synergistic—they interact—so that a certain amount of risk from smoking is not just added to a certain amount of risk from alcohol. The increase in risk is much more than you would get just from summing up the individual risks conferred by each separate factor. So there seems to be a synergy involved in increasing larynx cancer risk.

—KENNETH ROTHMAN, MD, *Bethesda*

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