

Detection of Viral Genes and Their Products in Chronic Neurological Diseases

Ashley T. Haase, MD,* J. Pagano, MD,† Byron Waksman, MD,‡ and Neal Nathanson, MD§

The suspicion that viruses are involved in multiple sclerosis (MS) now largely depends on epidemiological evidence. Numerous attempts to transmit MS have been unsuccessful, as have searches for viruslike particles or antigens. The April 1983 workshop "Detection of Virus Genes and Their Products in Chronic Neurological Disease," held at Woods Hole, Massachusetts, under the auspices of the National Institutes of Health and National Multiple Sclerosis Society, brought together fifty-six scientists from eight countries to reexamine the issue of viral infection in MS for two reasons: the new information on persistent viral infections and the subtle mechanisms that link infection to disease, and the newly available technical opportunities to penetrate these mysteries.

Using these instruments to full advantage depends on knowing what to look for, and there was considerable discussion of contemporary views of viral persistence *in vivo*. These are summarized in the Figure. Within the framework of the virus life cycle, there are four classes of mechanisms that, first, allow a virus to elude immune surveillance and, second, allow the host to survive for long periods. Both of these requirements must be satisfied in persistent infections. The most basic mechanism (type I) is analogous to lysogeny, with global repression of the viral genome such that the infected cell escapes detection and destruction because little if any antigen is present to focus the attack by immune and inflammatory cells. The slow infections *visna* and subacute sclerosing panencephalitis (SSPE) exemplify type I persistence. In types II and III surface antigenic targets are also greatly reduced, but by mechanisms that include antibody-mediated modulation of envelope components, and alterations in the synthesis or stability of the matrix polypeptide required for virus assembly. These mechanisms entrap the virus in the cell and place it beyond the reach of host defenses. Types II and III persistence also are exemplified by SSPE. In type IV persistence the final phase of virus

maturation involving host cell proteolytic cleavage of a protein essential for infectivity, such as the fusion protein of paramyxoviruses, fails to take place, and virus continues to be harbored in cells. Persistent infection of lymphocytes by this mechanism raises the possibility of episodic disease if these cells should enter the nervous system and gain access to the requisite proteases.

In type I persistence the only discernible evidence of infection is the viral genome and varying numbers of transcripts. In this situation hybridization methods are the appropriate means of investigation (Table 1). If infected cells are numerous, analysis of nucleic acid extracted from tissues by solid-phase methods will detect virus genes and integrated genomes. By using cloned probes from defined regions of the genome, additional information can be obtained about whether all or part of the genome is present and expressed. At the practical level the use of probes from small regions of the genome, or probes from reiterated regions of the genome, amplifies the signal and greatly enhances the sensitivity of the probe.

In many chronic infections there are only a few infected cells in the tissues. Methods that rely on extracted nucleic acids will fail to detect virus genes because of dilution. The method of choice in this situation is *in situ* hybridization of individual cells. Results obtained with MS and non-MS neural tissue illustrate the relative sensitivity of these methods: measles virus-like nucleotide sequences have been detected by *in situ* hybridization in a few cells in tissue sections, but not in nucleic acids extracted from the tissues.

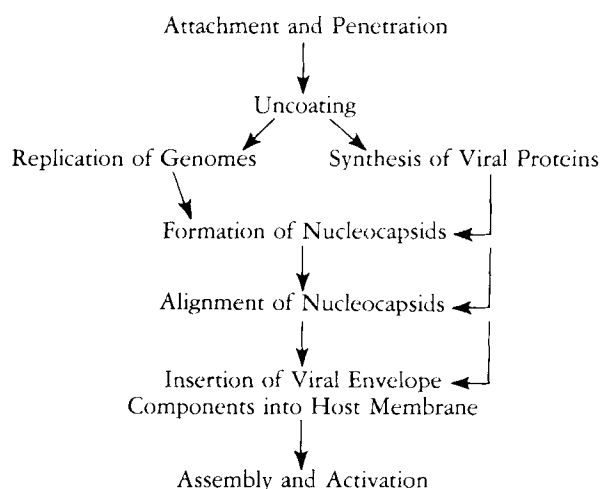
One limitation of *in situ* hybridization is the small area of tissue subjected to analysis. This problem of sampling can be overcome by "hybridization histochemistry," a technique in which the tissue is placed against x-ray film after hybridization to a radioactive probe. Darkening of the film serves as a guide to regions of the tissue that can be fruitfully examined microscopically, after coating the tissue with radioauto-

From the *Department of Infectious Disease, 4150 Clement St, Veterans Administration Medical Center, San Francisco, CA 94121, the †Cancer Research Center, University of North Carolina, Chapel Hill, NC 27514, the ‡National Multiple Sclerosis Society, New York, NY, and the §Department of Microbiology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Received June 27, 1983. Accepted for publication July 2, 1983.

Address reprint requests to Dr Haase.

GENERALIZED VIRUS LIFE CYCLE



graphic emulsion. Most hybridization techniques make use of radioactive labeled probes. Nonradioactive probes that can be visualized by fluorescence or by an enzymatically developed color reaction show great promise.

How are persistent infections related to disease? Table 2 indicates that in some cases the relationship is direct, e.g., demyelination as a consequence of lytic infection of the oligodendrocyte by coronaviruses or papovaviruses. With recombinant DNA techniques, in the case of herpes simplex virus it is now possible to define precisely the region of a virus genome directly responsible for neurovirulence. In other instances the link between infection and disease is not easily discerned: for example, if the virus causes dysfunction of cells that elaborate hormones with distant target organs, or if the virus sets in motion a pathological process whose effects extend beyond the boundaries of the initial infection. In the demyelinating phase of Theiler's virus infection of mice, the viral genome persists in glial cells, and the correlation between foci of inflammation, demyelination, and cells with viral antigen suggests that disease is related to a virus-induced immune attack on white matter. In other animal mod-

MECHANISMS OF PERSISTENCE

I. Decreased Synthesis and Expression of Viral Genome

II. Decreased Synthesis or Stability of Components Required for Alignment

III. Decreased Synthesis or Antibody Modulation of Envelope Components

IV. Failure to Activate Virus by Proteolytic Cleavage

Mechanisms of viral persistence in vivo.

els of demyelination, such as rats infected with coronavirus, the virus apparently sensitizes the animal to myelin basic protein, and infection leads to an autoimmune leukoencephalomyelitis.

The most difficult situations to analyze are those in which the virus initiates a pathological change but need not be present at the time of manifest disease, or in which episodes of disease follow introduction of virus from an extraneural reservoir. Such indirect pathogenic mechanisms make it virtually impossible to satisfy the molecular equivalents of Koch's postulates: constant association of a virus genome with disease, and in a location that plausibly accounts for the pathological findings. The discoveries of herpes simplex and measles virus genes in the brains of MS patients and control subjects can be interpreted as adventitious infection, or, alternately, as residual evidence of the source of an autoimmune process directed to white matter.

The cause of MS remains an enigma, but the conference ended with optimism, founded in the formidable powers of the new technology, and the successes of the past ten years in the analysis of the relationship among virus genes, host genes, and cancer.

Table 1. Experimental Strategies to Detect Viral Genes and Their Products

Method	Application
HYBRIDIZATION ASSAYS	
1. Probes: radioactive; non-radioactive; specific for all or part of the genomes; oligonucleotide and reiterated sequence probes; probes from libraries	Type I persistence; direct detection of viral genes and genomes
2. Population analyses on extracted nucleic acid a. Methods: solid-phase hybridization—"southern, northern, and dot blots" b. Information: whether all or part of the genome is present; whether genome is integrated; number, kind of ribonucleic acid species	Infected cells constitute a significant proportion of cells in the tissue
3. Single-cell analyses a. Method: in situ hybridization b. Information: whether all or part of the genome is present; type of cell; localization in the cell; number, kind of ribonucleic acid species	Infected cells constitute only a small proportion of cells in the tissue
4. Combined analysis a. Method: hybridization histochemistry b. Information: combination of 2 and 3	Screening tissues
PROTEIN ASSAYS	
1. Reagents: monoclonal antibody; site-specific antibody to peptides	Types II–IV persistence; analysis of mechanisms
2. Methods a. Radioimmunological: precipitation and "western" blots b. Combination assays: in situ hybridization; cells probed by fluorescence or immunoperoxidase methods	

Table 2. Mechanisms of Disease in Persistent Viral Infections of the Central Nervous System

Mechanism	Example
DIRECT	
1. Cell killing	Lytic coronavirus infection of oligodendrocytes causes demyelination
2. Cell dysfunction	Lymphocytic choriomeningitis virus infection of pituitary cells diminishes production of growth hormone and causes runting
INDIRECT	
1. Immune and inflammatory cell attack directed toward a. Viral antigens b. Shared determinants on viral antigens and host antigens c. Host antigens	Demyelination in Theiler's virus infection at sites of inflammation near cells with viral antigen Monoclonal antibody to the P protein of measles virus cross reacts with neurofilaments Coronavirus infection of rats sensitizes the animals to myelin basic protein
2. Hit and run	Cells transformed by herpes simplex virus may maintain their transformed state without discernible evidence of viral genomes
3. Extraneural reservoir of infection with episodic disease	Peripheral neuropathy in Marek's disease mediated in part by infiltrating lymphoblastoid cells transformed by virus Persistent infection of lymphocytes by measles virus, frequently mutant; replication in activated cells, or after decrease in interferon levels Persistent infection of macrophages by visna virus; isolation of antigenic variants