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Editorial

Human Retrovirus and Multiple Sclerosis

Multiple sclerosis (MS) is a chronic disorder of the central nervous system characterized pathologically by multifocal regions of perivascular inflammation, demyelination, and gliosis and clinically by a relapsing or chronic disease course (or both). This inflammatory pathologic picture has prompted the postulate that immune mechanisms induced by autoantigens or viral infection mediate the tissue injury. This postulate is supported by epidemiologic data that indicate a 30 to 50% disease concordance rate among identical twins, an overrepresentation of disease in various racial groups correlated with particular major histocompatibility complex gene products, a linkage of susceptibility with certain T-cell receptor genes, and the suggestion that migration beyond a critical age results in retaining the risk rate of the country of origin. Animal models of chronic demyelination induced by autoantigen sensitization (experimental allergic encephalomyelitis), by lytic infection of oligodendrocytes (neurotropic mouse hepatitis [JHM] virus, canine distemper), and by viral-induced immune mechanisms (Theiler murine encephalomyelitis virus, JHM virus in rats) provide evidence that such viral or immune mechanisms (or both) can induce chronic demyelinating disease of the central nervous system.

The search for either an autoantigen to which patients with MS are specifically sensitized or a persistent MS-associated viral infection has been ongoing for decades in concert with advances in biologic concepts about persistence of virus, viral-immune interactions, neural-immune interactions, and mechanisms of autoimmunity and with advances in technology to conduct such studies. Recent examples of technologic advances relevant to MS include neuroimaging techniques that allow following the evolution of lesions in patients with MS, the isolation and maintenance of myelin-reactive T-cell lines from patients with MS and control subjects, novel tissue culture and cell biologic approaches to myelinating cells (oligodendrocytes), and the molecular biologic analyses of gene products critical for viral persistence. The search for unique viruses in MS has included indirect (serologic) and direct (immunofluorescence, electron microscopy, tissue culture, in situ hybridization, molecular cloning) techniques. Candidate viruses have included double-stranded DNA viruses (for example, herpes simplex) and single-stranded RNA viruses (paramyxoviruses—parainfluenza, measles, coronavirus). None has met the requirements of Koch's postulates with regard to MS, although some are able to induce the presumed immunopathogenic uniphasic post-infectious encephalomyelitis syndrome in humans or demyelination of the central nervous system in animals.

The report by Prayoonwiwat and associates in this issue of the *Proceedings* (pages 665 to 680) addresses the potential role of retroviruses in MS. The term "retrovirus" is derived from the replication cycle of these enveloped single-stranded RNA viruses, which occurs through a double-stranded DNA intermediate synthesized by an RNA-dependent DNA polymerase (reverse transcriptase). A sheep retrovirus, visna, has long been known to persist within the nervous system and to induce an inflammatory chronic demyelinating disease, although the precise basis for the tissue injury—direct cytopathic or indirect immunopathogenic—remains to be determined. In 1985, the initial reports indicated that, based on serologic evidence, a progressive myelopathic syndrome endemic to tropical regions of Central America—that is, tropical spastic paraparesis (TSP)—and to specific regions of Japan—that is, human T-cell lymphotropic virus type I (HTLV-I)-associated myelopathy

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(HAM)—was associated with HTLV-I retrovirus infection. Subsequently, HTLV-I was directly identified in and isolated from lymphoid cells of the cerebrospinal fluid of such patients.

The HAM-TSP syndrome pathologically is characterized by inflammation within both white and gray matter of the spinal cord and degeneration of white matter including myelin and axons, usually in a symmetric distribution, with lateral columns most affected.[1] The inflammation seems to be the primary lesion. Prominent neuropathologic changes also include fibrous thickening of the adventitia of blood vessels and prominent microglial accumulation. The extent of the degeneration of the white matter is distinct from the pathologic findings in MS. To date, direct viral persistence within primary cells of the central nervous system has not been substantiated although HTLV-I infection of adult glial cells in vitro has been reported.[2] The neurologic syndrome develops in less than 1% of HTLV-I-infected persons, consistent with the concept of an autoimmune process associated with a persistent infection of T lymphocytes rather than a direct infection of tissue in the central nervous system. Jacobson and colleagues[3] demonstrated that only the affected persons have class I major histocompatibility complex-restricted cytotoxic T cells directed against the HTLV-I tax protein encoded in the regulatory region pX. These T cells could represent a unique response to the virus by neurologically affected persons or could reflect the response in patients with high viral loads.

The specific question of prototype HTLV-I being the cause of, or at least associated with, MS was raised by initial reports of increased serum and cerebrospinal fluid titers of HTLV-I antibodies in patients with MS in comparison with "control subjects."[4] Numerous subsequent studies attempted to take into account such issues as selection of patients and control subjects and technical aspects of the assays used, such as the HTLV-I antigen preparations. The final consensus was that no significant differences were evident in serum antibody titers when patients with MS were compared with control subjects, particularly patients affected by other autoimmune diseases (as reviewed by Prayoonwiwat and co-workers).

The initial serologic studies were followed by reports that HTLV-I sequences could be detected in DNA extracted from blood, cerebrospinal fluid lymphocytes, or tissue from the central nervous system itself from patients with MS.[5] Most of these studies, as in the current report, used the polymerase chain reaction technique to amplify viral DNA sequences. A clear consensus is that prototype HTLV-I is not reproducibly detectable in MS tissues.

The reports by Prayoonwiwat and associates, Ehrlich and colleagues,[6] and the MS-National Institutes of Health consensus conference[7] indicate some of the clinical and technical laboratory issues that may have contributed to the initial controversy about HTLV-I in MS and that should be kept in mind as the search for unique retroviruses or other viruses in MS continues. The major controversy about HTLV-I in MS relates to "false-positive" results because "false-negative" findings could not be otherwise confirmed. Patients have been selected for study on the basis of clinical criteria, many having progressive disease. Clinical descriptions of HAM-TSP indicate that this entity can mimic progressive MS, and occasional patients have brain-stem or optic nerve lesions.[1,8] Magnetic resonance imaging is also not invariably able to distinguish MS and HAM-TSP; lesions of the cerebral white matter have been detected in both conditions. The HTLV-I serology in the cerebrospinal fluid should distinguish the two entities, although claims have been made for serologically negative, polymerase chain reaction-positive cases in endemic areas.[6] The issue of case selection, including clinical course, geographic and other epidemiologic considerations (transfusions, sexual contacts), neuroimaging, and blood and cerebrospinal fluid serologic analyses, should receive as much attention as the laboratory technical details and indeed likely account for some of the previous controversies.

With regard to the laboratory technical details, "false-positive" results are again central to the question, "Does HTLV-I cause MS?" The HTLV-I transcriptional unit contains at least

three functional genes—*gag*, *pol*, and *env*—as well as genes that encode regulatory proteins. The genome is flanked by a long terminal repeat in the provirus, which is usually thought to act as a promoter-enhancer region. Several studies have concluded that, under high stringency conditions, with use of the polymerase chain reaction gene-amplification technique applied to lymphocyte- or central nervous system-derived DNA, one does not obtain hybridization signals that correspond to each of the functional HTLV-I genes or long terminal repeat regions, in contrast to data from HAM-TSP cases; thus, prototype HTLV-I is excluded as a consistent feature of MS. Technical laboratory causes of "false-positive" results derive in large measure from the highly sensitive nature of the polymerase chain reaction technique. Causes include contamination of samples directly during extraction, plasmid contamination, or aerosol contamination. Among HTLV-I isolates from different HTLV-I-infected persons, sequence differences are evident; concern about laboratory contamination is thus raised when identical gene sequences are amplified from blood samples from different patients, such as in the original six patients analyzed by Reddy and associates,[5] all of whom had identical *env*-gene sequences. When other investigators studied these same donors that had been analyzed by Reddy and colleagues, negative results were reported.[9] In several studies, including the current one that used nested primers, hybridization signals on a given sample are obtained with some but not all of the primers designed to amplify HTLV-I genes. These sporadic signals from control and disease donors suggest that the signals are not derived from HTLV-I and most likely represent endogenous retroviral sequences present in the human genome (see subsequent discussion). The studies of HTLV-I and MS illustrate, to the credit of those who have participated, how collective efforts and interchange can rapidly lead to important results, whether positive or negative.

Endogenous retroviruses are proviral sequences that are vertically transmitted through the germline, in contrast to the horizontal transmission of "exogenous" virus between persons. The human genome is host to thousands of copies of retroviral proviruses—some originating before the divergence of primates and some reflecting relatively recent integration. Most of the proviral-like sequences in the human genome are defective, lacking large regions necessary for viral replication. Many relatively complete genomes have undergone mutation by the acquisition of stop codons in open reading frames, precluding any expression of intact viral proteins. Putative retroviruses and reverse transcriptase activity, however, have been detected in serum samples from patients with breast cancer, in non-HTLV-I- or HTLV-II-related leukemias and lymphomas, and in normal human breast milk, placenta, and serum. These observations suggest that at least some endogenous retroviruses are still capable of coding full viral particles.

The potential influence of retroviruses on T-cell repertoire and T-cell reactivity has recently been the focus of attention because of the observations that self-superantigens in mice can be coded by retroviruses.[10,11] Superantigens are molecules that, in conjunction with cell surface class II major histocompatibility complex molecules, are able by interaction with a variable domain of the β subunit of the T-cell receptor to activate an entire class of T cells that bear a specific allele (for example, Vβ14), independent of the antigen binding site. The functional consequences of such interactions depend on the location (periphery versus thymus) of the interaction and the developmental stage of the animal. Interaction in the thymus during development would result in depletion of T cells that bear specific T-cell receptor V-region encoded domains and thus shape the subsequent T-cell repertoire of the patient. Interaction outside the thymus (systemic or intrathecal compartments) could result in T-cell activation. In humans, various microbial superantigens (staphylococcal enterotoxin, *Mycoplasma*) can induce such responses.

Could endogenous retroviruses contribute to development of apparent autoimmune disease, including MS? It seems unlikely that intact

retroviruses could be causing lysis or direct dysfunction of oligodendrocytes because such viral particles are not reproducibly found in the lesions of the central nervous system. Several possible models could be considered whereby endogenous viral proteins, particularly *env* glycoproteins, could underlie immune-mediated injury to the myelin of the central nervous system in the absence of complete viral particles. Because antigens expressed during development cause ablation or tolerance of T-cell clones directed against them, one might postulate that immunoreactive viral proteins would be expressed late, possibly because of alterations in *cis* elements (for example, aging-related loss of DNA methylation[12]) or transacting factors (for example, up-regulation with steroid hormones in puberty). *Env* protein, or other viral proteins, could be expressed by oligodendrocytes and thereby trigger destruction by sensitized T cells. Common antigenicity between viral proteins, or posttranslational modifications of these proteins, and oligodendrocytes or their myelin membranes could produce immunologic cross-reactivity—that is, molecular mimicry. We have also proposed that antibodies directed against viral antigens that share immunoglobulin superfamily domains with human T cells could activate cells in the central nervous system by acting as a mitogen.[13] Low complement and ineffective reticuloendothelial function in the brain would favor a "mitogenic" over a lytic T-cell response, with demyelination occurring as a bystander effect. The presence of endogenous superantigens coded by endogenous retroviruses, as discussed in the foregoing material, suggests a further means whereby non-antigen receptor-mediated triggering of the T-cell receptor could activate cells in a "mitogenic" manner, with subsequent bystander demyelination.

The existent data about prototype HTLV-I as the cause of MS seem to have placed this issue in permanent remission. The emerging data on the influence of endogenous and exogenous retroviruses on selection of immune repertoire and immune activation suggest that the potential contribution of retroviruses to human autoimmune diseases, including MS, should, however, remain an active area of investigation.

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