Multiple sclerosis

Relationship to a retrovirus?

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E. PREMKUMAR Reddy and colleagues at the Wistar Institute in Philadelphia and the University of Lund in Sweden, in the 27 January issue of *Science*[1], describe the identification of DNA sequences corresponding to human T-lymphotropic virus (HTLV-I) in the peripheral blood mononuclear cells of six Swedish multiple sclerosis patients. They also describe their failure to find similar sequences in all but one of twenty healthy controls (ten Swedish and ten North American). Some but not all of the patients are reported to have antibodies against the virus in their blood.

This report is an extension of studies published by the same group 3 years ago[2] in collaboration with Robert Gallo's group at the National Cancer Institute. The new work[1] involves the use of the powerful polymerase chain reaction (PCR), which detects and amplifies low concentrations of viral material, allowing molecular cloning and sequencing of the amplified DNA. The report has, not surprisingly, caused a flurry of excitement in the media, because it suggests a relationship between a retrovirus related to HTLV-I and multiple sclerosis. It does not imply that the retrovirus is the cause of the disease, and the authors do not claim this. But it is possible that these findings will stimulate attempts to develop preventive viral vaccines or therapeutic investigations with drugs which arrest retroviral replication, such as those being developed for the distantly related AIDS virus.

Reddy and collaborators used typical multiple sclerosis patients in their new study[1], many of whom had exacerbating–remitting disease. These were clearly distinct from cases of the slowly progressive, multiple sclerosis-like disease tropical spastic paraparesis, or HTLV-I-associated myelopathy, which is widely distributed in Africa, the Caribbean area and southern Japan. This disease is clearly associated with, and probably caused by, HTLV-I, and clear-cut viral isolation has now been reported by several groups[3,4].

DNA sequences corresponding to both the *gag* and *env* regions of the HTLV-I genome were identified in peripheral blood mononuclear cells from all six multiple sclerosis patients studied by Reddy and collaborators[1]. The results seem persuasive because of the sharp distinction between patients and controls; the concordance of the findings with two different retroviral genes; and the sequencing data provided. At least one other group of investigators, in a study which will shortly appear[5], has succeeded in demonstrating HTLV-I-like DNA sequences in the *pol* and *env* regions in some multiple sclerosis patients but not in normal controls. But when this group used a *gag* primer similar to that used by Reddy *et al.*, all of a series of 21 multiple sclerosis patients, 35 normal controls and 10 cord blood samples gave positive results.

The most interesting possibility raised by the results from both groups[1,5] is that an exogenous retrovirus related to HTLV-I may have a direct causative role in multiple sclerosis. The failure of most laboratories to find antibodies reactive with this virus, and the discrepant antibody finding of Reddy *et al.*[1], speak against this. Another possibility is that Reddy *et al.* are looking at 'endogenous' retroviral DNA sequences, which are widespread in human and other mammalian germline DNA[6]. For some viruses, the complete structure, including *gag*, *pol* and *env* sequences, is often maintained; intact infectious virus is usually not found. At least five known families of retroviruses have been demonstrated in the normal human genome. When supersensitive techniques like the PCR are used, retroviruses resembling HTLV-I can be found in many individuals[6]. But if endogenous retroviral sequences account for the multiple sclerosis results of Reddy *et al.*[1], why were 19 of the 20 controls negative? Results obtained with the PCR technique vary greatly from day to day, and Reddy *et al.*[1] do not report how often they carried out the tests in the multiple sclerosis samples and the controls or even if they ran the multiple sclerosis and control samples concurrently. There is therefore some uncertainty whether the possibility of endogenous sequences is excluded.

Another spectre haunts those who use the PCR technique — the possibility that the test system is contaminated with retroviruses already present in the laboratory. Some take extreme precautions to deal with this possibility, carrying out the PCR in a different laboratory or building than that in which cell separations are done. Contamination can be excluded when the sequences found differ at many positions from all possible contaminants. However, the sequences described by Reddy and colleagues in the multiple sclerosis samples differ only slightly from the comparable sequences of the HTLV-I (derived from patients with tropical spastic paraparesis) studied earlier in the same laboratory. Because the PCR is not an error-free technique, this may be a source of concern.

It is regrettable that the investigators failed to test their techniques on blood cells from patients with diseases related to multiple sclerosis, such as rheumatoid arthritis or juvenile diabetes, or even on mitogen-stimulated normal cells, as the circulating monocytes and lymphocytes of both main subsets are markedly activated in multiple sclerosis, as well as in these other conditions[7]. Cell activation by, for example, phorbol esters results in enhanced retroviral gene expression[8]. Although any possible effect of such activation on the PCR staining of DNA is conjectural, it would be reassuring to know that tests for HTLV-I on activated blood mononuclear cells of such controls were consistently negative.

The pathogenesis of the characteristic inflammatory destructive lesions of multiple sclerosis, rheumatoid arthritis, insulin-dependent diabetes, chronic thyroiditis and other similar diseases is explained by many as an immunological reaction to tissue antigen triggered by viral infection (measles, rubella, Epstein–Barr virus) in early life in genetically predisposed individuals[7,9]. This view is based on epidemiological evidence and the study of autoimmune animal models (see my earlier News and Views article[9]), and receives strong support from recent studies of genes associated with susceptibility to multiple sclerosis and of those governing recognition of myelin antigens by T cells. Perhaps an autoimmune reaction can trigger the local expression of exogenous or endogenous retroviral genes, which make a secondary contribution to lesion pathogenesis, but this remains little more than a speculation.

To conclude on a sobering note, one should recall that more than 20 viruses have been incriminated as the possible cause of multiple sclerosis since 1946[10]. In twelve instances, the presumed causative virus was actually isolated from multiple sclerosis patients. Yet none of these candidates has stood the test of time. This underlines the need for further studies in other laboratories to confirm the findings of Reddy *et al.*[1] and for control studies in other neurological and autoimmune diseases. Putting these cautions to one side for a moment, the new findings are among the most interesting to be published in recent years in this difficult field. □

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