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Infectious etiology in multiple sclerosis: the debate continues

Francisco González-Scarano and Bert Rima

Multiple sclerosis (MS) affects over 300 000 Americans, 85 000 Britons and perhaps in excess of two million people worldwide, and has long been suspected to have an infectious etiology. However, despite intensive investigation, no single infectious agent or group of agents has been identified. 'Demonstrating Infectious Cause: Viral and Bacterial Infections in MS and Related Disorders' brought together immunologists, virologists, epidemiologists and clinicians to review the current knowledge about the role of infectious agents in MS, and to identify key concepts and questions for research.

Nineteenth-century descriptions of the neuropathology of MS emphasized the prominent infiltration of leukocytes and other inflammatory cells into its characteristic central nervous system (CNS) lesion, which is now called a plaque. Although it is now well accepted that inflammatory responses often have non-infectious causes, there are a number of clues that point towards an exogenous, possibly infectious, factor or factors as an important component of MS. For example, studies of twins with MS indicate that monozygosity is associated with a disease concordance of ~35% which, although suggesting a strong genetic component (the concordance for dizygotic twins is in the order of 4%), also indicates that the disease is influenced by additional, possibly environmental, factors. Furthermore, there is a strong geographical influence on MS prevalence: the rates range from 150–250 per 100 000 in northern Europe and in the northernmost USA, whereas in southern Europe the prevalence drops to ~40 per 100 000, similar to the rates in the southern USA.

'Demonstrating Infectious Cause: Viral and Bacterial Infections in MS and Related Disorders' was held in Brighton, UK, 23–25 August 1999.

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Remarkably, a large number of epidemiological studies have shown that individuals maintain the 'signature' incidence associated with the geographical area in which they spent their youth, even after migrating from an area of high incidence to one of low incidence, or vice versa. This has been interpreted as evidence that exposure to a microorganism prior to adulthood predisposes people to the development of MS (Ref. 1).

The most persuasive evidence that foreign antigens are associated with MS, however, is the indication of a humoral immune response within the CNS. Detectable as 'oligoclonal' bands in electrophoresed samples of concentrated cerebrospinal fluid (CSF) proteins, antibodies produced by plasma cells within the CNS are usually seen in infectious diseases such as syphilis or AIDS, where they are directed against antigenic components of the invading microorganism. Their presence and persistence in MS (Ref. 2) suggests that a chronic infection underlies the clinical syndrome, although it is also possible that these antibodies are directed against as-yet-unidentified self-antigens. Additionally, analysis of tissue samples from around

the plaques themselves also indicates antibody production and deposition. Most investigators believe that identification of the antigens against which these antibodies are directed will provide important information about MS, perhaps even identifying its cause. Although there were calls from conference 'break-out' sessions for better stratification of MS patients in epidemiological studies, the consensus of studies pointing in the direction of an environmental cause, as well as the evidence from the oligoclonal immune responses, convinced the majority of delegates that MS is likely to be caused or triggered by an infectious agent.

The infectious agent: known or unknown?

The majority of studies concerning specific agents in MS are based on the assumption that the microorganisms persist in the patient, probably in the CNS but perhaps in other organs. A number of new technologies such as representational difference analysis (RDA), differential display, consensus PCR and phage display are now available for identification of unknown or difficult-to-culture organisms.

With that in mind, Donald Gilden (University of Colorado, Denver, CO, USA) has adapted phage-display technology to analyse the antigen specificity of oligoclonal bands by extracting the RNA surrounding an MS plaque and amplifying the immunoglobulin heavy and light chains using consensus primers. After cloning into an expression vector, the immunoglobulins are displayed on the coat of a bacteriophage. These immunoglobulins can then be specifically amplified, and the antigen against which they are directed can subsequently be identified.

Gilden has already achieved success using this approach for at least one chronic infection caused by measles virus (subacute sclerosing panencephalitis; SSPE). His laboratory is now directing its attention to the MS lesions and the CNS areas where the CSF is produced.

Other presentations concerning molecular technologies gave the overall impression that the new technologies have not yet been applied in a systematic fashion to pathologically well defined MS tissue, and that there was room for more work using these approaches.

Some investigators felt that the agent that induces MS has already been identified. Subramamiam Sriram (Vanderbilt Stallworth Rehabilitation Hospital, Memphis, TN, USA) presented data indicating that the CSF of 97% of MS patients (compared with 18% of patients with other neurological diseases) contains genomic material from the bacterium *Chlamydia pneumoniae*, as shown by PCR amplification. Sriram was also able to culture *C. pneumoniae* from the CSF of MS patients. Furthermore, oligoclonal bands from MS CSF were adsorbed by incubation of the fluid with a *C. pneumoniae* antigen, but not with antigens from herpes simplex or measles viruses. These data support his view that the immune response in those patients was directed against *C. pneumoniae*. Sriram also suggested that a therapeutic trial with antibiotics directed against *C. pneumoniae* might be required to demonstrate a causal association between this microorganism and MS.

Konstance Knox (Institute for Viral Pathogenesis, Milwaukee, WI, USA) presented equally compelling data supporting earlier suggestions³ that human herpesvirus six (HHV-6) is associated with MS. Immunohistochemical analysis of MS plaques has revealed a concentration of an HHV-6 antigen in oligodendrocytes, the CNS cells responsible for the formation and maintenance of myelin (myelin destruction is a key component of the MS lesion). Knox and collaborators found HHV-6 expression in 17 out of 19 patients with 'active'

disease, compared with only three out of 23 patients with 'inactive' MS. There were also dramatic differences in viremia. Twenty seven out of 41 patients with MS had active HHV-6 viremia, compared with none of the 61 controls. Other investigators presented data supporting potential roles for human endogenous retroviruses (Herve Perron, Biomerieux SA, Lyon, France), and delayed exposure to Epstein-Barr virus (Tove Christensen and Sven Haar, Aarhus University, Aarhus, Denmark) as potential triggers^{4,5}. However, as with many such reports, in most individuals there is evidence of prior or current infection with these agents whether or not they have MS, and there are no more-definitive tests such as viremia or analysis of oligoclonal bands to differentiate patients with MS from those with other diseases. Further research will be needed in order to clarify and reconcile these potentially conflicting findings, as several presentations outlined an association with a specific agent in over half of the cases.

Microbial hit-and-run?

It is entirely possible that multiple microorganisms are each responsible for a small proportion of this disease, perhaps indirectly, by inducing immune responses that result in an immune attack on the CNS, without persisting in the affected patient. Several animal models of virally induced demyelination, particularly those associated with murine coronaviruses or with Theiler's murine encephalomyelitis virus (TMEV), support such an idea. In these models, demyelination is related to the presence of an identified virus within the CNS; in TMEV-mediated disease, the extent of demyelination depends not only on viral load but also on a locus on mouse chromosome 11 (Michel Brahic, Institut Pasteur, Paris, France). Although an intact immune system is necessary for the disease to manifest in its full form, which arm of the immune response is most critical varies from model to model, and even within models, apparently depending on the viral strain.

Demyelination after TMEV infection of mice transgenic for a human leukocyte antigen (HLA) was shown to be modulated by the HLA transgene (Moses Rodriguez, Mayo Clinic, Rochester, MN, USA), a finding that might relate to the preponderance of certain HLA subtypes in MS. Interestingly, by careful injection, foreign antigens can be introduced into the brain of mice and not be 'seen' by the immune system until after a subsequent peripheral challenge, which leads to detection and commencement of an immunopathological process (Hugh Perry, University of Southampton, Southampton, UK).

Mechanisms of autoimmunity?

Whether or not a single agent is indeed responsible for this disease, there are multiple ways in which an infectious agent could be associated with, or trigger, MS. In some of these, the microorganism might no longer be present by the time the disease manifests but, for example, it affects the CNS by changing the intrathecal cytokine environment and promoting pro-inflammatory reactions or by stimulating autoreactive T cells. Matthias Von Herrath (Scripps Clinic, San Diego, CA, USA) indicated that, in an autoimmune diabetes model, the growth kinetics of different strains of lymphocytic choriomeningitis virus determined whether the disease was exacerbated by a second peripheral challenge or whether this was, in fact, protective. Balances between immune reactions also produce different responses in Borna virus infection in rats (Luther Stitz, Institute of Animal Health, Tübingen, Germany). One of the more popular arguments has been that a microorganism could stimulate an autoimmune response by mimicking an endogenous antigen (antigenic mimicry) (Uwe Liebert, University of Leipzig, Leipzig, Germany; Henry McFarland, National Institutes of Health, Bethesda, MD, USA); circumstantial evidence indicates that such a mechanism could lead to the development of autoimmune, reactive T-cell clones^{6,7}. Alternatively, an exogenous agent could act as an

adjuvant, perhaps by incorporating a self-antigen into its structure, much like retroviruses can incorporate cellular proteins into their envelopes⁸. Steve Miller (Northwestern University Medical School, Chicago, IL, USA) presented data to indicate that only antigen-presenting cells from TMEV-infected mice with pre-existing myelin damage and not from acutely infected or control mice were able to present proteolipid apoprotein (PLP) epitopes to specific T-helper type 1 cell lines.

Taken together, the presentations and discussion at the Brighton conference summarized an impressive amount of information, leading to the conclusion

that there are indeed exogenous agents involved in the development of MS. However, it is also clear that at this point there are insufficient data to identify a single culprit, and future research will be directed at replicating some of the most promising findings to date, and at understanding the possible pathogenic mechanisms by which foreign antigens could trigger an immune attack on the CNS.

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Letters

Why is IFN- γ insufficient to control tuberculosis?

Immunological control of *Mycobacterium tuberculosis* infection is complex and multifaceted. As indicated in the review by Murray¹, both CD4⁺ and CD8⁺ T cells are essential to control tuberculosis (TB) in mice^{2–5}, and recent data support a role for both types of T cells in human TB resistance^{6–8}. Macrophage activation, which is likely to occur within a granuloma, is the key to controlling TB. Granulomas consist of macrophages and lymphocytes, but the role(s) of each cell type, the types and kinetics of the cytokines produced by different cell populations, and the evolution of the granuloma remain poorly understood. Murine studies have revealed essential roles for certain cytokines, including interferon γ (IFN- γ), interleukin 12 (IL-12) and tumour necrosis factor α (TNF- α). However, there is little data regarding the importance of these cytokines in human TB. Humans with mutations in the genes encoding IFN- γ or IL-12, or their receptors, often succumb to mycobacterial infections other than TB (Ref. 9). Although it is probable that these cytokines are significant in human TB, their relative importance, specific roles and cellular targets remain less clear.

Most patients infected with *M. tuberculosis* have CD4⁺ T-cell responses that are expected to be effective in activating macrophages to kill intracellular mycobacteria

(e.g. IFN- γ production). However, because most infected individuals harbor the organism for many years, perhaps for a lifetime, this response is apparently not completely effective in eliminating virulent *M. tuberculosis*. In fact, *in vitro*, it has been difficult to demonstrate IFN- γ -induced killing of *M. tuberculosis* within human macrophages, although similarly activated macrophages eliminate other intracellular pathogens. A recent study has shed some light on this phenomenon¹⁰. It has been shown that macrophages infected with *M. tuberculosis* are resistant to the effects of IFN- γ , as demonstrated by the inability of *M. tuberculosis*-infected macrophages to kill a secondary intracellular parasite, *Toxoplasma gondii*. Additionally, IFN- γ induction of Fc γ R1 is blocked by *M. tuberculosis* infection. All early steps in the IFN- γ signal transduction pathway are unaffected in *M. tuberculosis*-infected cells. However, the infection dramatically inhibits the association of the transcription factor STAT1 (signal transducers and activators of transcription 1) with the transcriptional coactivators CBP [cAMP-response-element-binding protein (CREB)-binding protein] and p300, an association that is essential for IFN- γ -induced transcription. Live bacteria are not necessary for this effect, as it can be reproduced using cell walls and it does not appear to

be mediated by lipoarabinomannan. Neutralization of transforming growth factor β (TGF- β) or IL-10 did not reverse the effects of *M. tuberculosis* infection, suggesting that these cytokines are not the only contributors to macrophage deactivation. This phenomenon might also occur in mice, because mice do not eliminate *M. tuberculosis*, even though a strong immune response involving both CD4⁺ and CD8⁺ T cells and production of IFN- γ , TNF- α and other cytokines is present.

M. tuberculosis is an enormously successful pathogen that has evolved numerous mechanisms for evading elimination by the host immune response. We are still in the process of learning which immune responses are important in controlling *M. tuberculosis* during the acute and latent phases of the infection. In most cases, the host maintains control of the infection, but occasionally the bacterium gains the upper hand. Dissecting these interactions at an immunological level in both animal models and human systems remains the challenge for TB research in the future. The development of better vaccines ultimately depends on our increased understanding of the host–pathogen interaction.

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