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Are Virus Infections Triggers for Autoimmune Disease?

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Abstract

Viruses have been implicated in the initiation, progression, and exacerbation of several human autoimmune diseases, including multiple sclerosis. However, no single virus has been demonstrated as the etiologic agent. Multiple different infections may be involved, first in priming the immune system for autoimmunity and then in triggering the actual disease. A model based on experimental allergic encephalomyelitis, an animal model of multiple sclerosis, has been developed, which shows that an initial early infection with a virus having molecular mimicry to self-epitopes can prime for disease that occurs after a subsequent non-specific immunologic stimulus, such as a different infection. The role of multiple infections in the development of autoimmune disease may explain why no one virus has been implicated.

What are Autoimmunity and Autoimmune Disease?

Autoimmunity is the process whereby the immune system, which normally protects the host from microorganisms, malfunctions or is fooled and attacks "self," destroying host tissues and leading to immunopathology. Both genetic and environmental influences are important in the induction and progression of autoimmune diseases (1). Autoimmune diseases are classified as either organ specific or systemic, depending on whether the autoimmune responses are against antigens confined to a particular organ or widely distributed throughout the body (2).

Viruses have long been thought to trigger autoimmunity, thereby leading to autoimmune disease. Several mechanisms have been proposed to explain virus triggers of autoimmune disease. One mechanism is molecular mimicry, whereby the immune response to the virus cross-reacts with self, resulting in

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autoimmunity (3-8). Another is epitope spreading, in which an initial immune response to a single epitope of a self-protein progresses to an immune response to other self-epitopes of the same protein (intramolecular spreading) and then to other self proteins (intermolecular spreading) (8-11). A related mechanism is bystander activation, whereby virus infection causes inflammation and tissue damage in the surrounding area, thus releasing host antigens, which together with induced immune host factors initiate autoimmunity (12,13). It has been proposed that multiple virus infections may be required for the development of autoimmunity. The initial infection may prime the immune system, while a subsequent infection with the same or antigenically related virus may trigger the autoimmune reaction (14).

Approximately 40 autoimmune diseases exist in humans (1). Organ-specific autoimmune diseases include insulin-dependent diabetes mellitus (IDDM, Type 1), rheumatic heart disease, and thyroiditis. IDDM is the autoimmune disease that occurs after the destruction of the pancreatic β cells by T lymphocytes (15). Characteristics

of IDDM are insufficient insulin secretion and the inability to regulate glucose homeostasis (16). Rheumatic heart disease is the autoimmune disease affecting various regions of the heart. It can follow streptococcal infection in which there are cross-reactive epitopes between the streptococcus and heart tissue (17). Thyroiditis is the autoimmune disease that occurs after the destruction of thyrocytes (18). Characteristics of thyroiditis are defective thyroid cell function and lymphocytic infiltration of the thyroid with or without the presence of a goiter (18).

Systemic autoimmune diseases include systemic lupus erythematosus, which affects the joints, kidneys, skin, mucous membranes, blood, and the cardiopulmonary and central nervous systems (CNS), where much of the pathology is caused by autoantibody

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immune complexes (19). Many more examples of human autoimmune diseases exist, which differ in both the organ affected and in the extent of production of autoreactive antibodies and/or autoreactive T cells (20-22).

What Is MS?

Multiple sclerosis (MS) is the most common human demyelinating disease. MS affects approximately 1 in 1,000 individuals in the United States (23). The disease is usually diagnosed in persons between the ages of 20 and 40 and affects women twice as often as men. It is an autoimmune disease of the CNS characterized by inflammatory demyelinating lesions that coalesce into large plaque-like regions within white matter (24). The main target in MS is thought to be the oligodendrocyte, the cell that forms myelin in the CNS. The lesions can appear in the optic nerve, brainstem, spinal cord, and periventricular white matter and can be multifocal in time and space. The autoreactive cells thought to be involved in lesion formation in MS are T cells (22).

The clinical course of MS varies from individual to individual. The clinical course of MS can be classified into four forms: relapsing-remitting (RR), primary progressive (PP), secondary progressive (SP), and progressive relapsing (PR) (25). RR-MS consists of disease relapses with full recovery or a residual deficit upon recovery but no progression between the relapses. PP-MS shows progression continuously from the onset. SP-MS is initially RR disease that is followed by progressive disease. PR-MS shows progression from the onset, with acute relapses and progression between the relapses.

What Is The Relationship Between Infections and MS?

Contrary to what would be expected because of its autoimmune nature, epidemiological studies point to a viral or microbial infection as the etiological agent for MS (26). After contact with
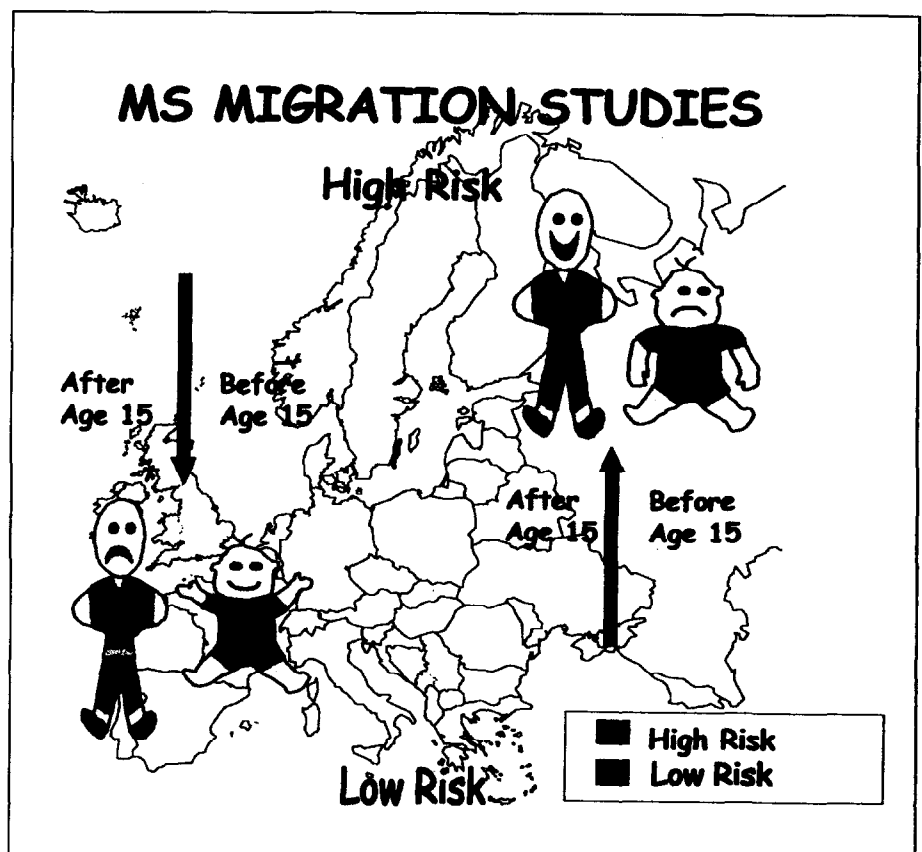

Figure 1. Overview of migration studies. Travel from a high-risk zone to a low-risk zone in the world after age 15 results in the individual keeping the high-risk phenotype, whereas migration before age 15 results in the individual acquiring the risk associated with the place of residence. The reverse is also true (28).

North Americans or Europeans, isolated populations with no reported cases of MS demonstrated an initial wave of MS followed by secondary cases appearing in small epidemics (27). As the distance from the equator increases, the risk of MS increases. Migration studies demonstrate that individuals moving between high- and low-risk areas for MS carry the risk of the area *from* which they move, if they move after age 15, but acquire the risk of the area *to* which they move if they move before age 15 (Fig. 1) (28). These studies suggest that exposure to or repeated infections with an agent before puberty contribute to determining an individual's risk for MS.

Viruses have long been suggested to

be the cause of MS. Viruses have been isolated directly from MS patients, and antibodies to viruses have been detected in them; however, to date, no single virus has been identified as the causative agent (29). On the other hand, exacerbations of MS have been correlated with viral infections (30-33).

Several animal models of MS are based on viral infections; for example, canine distemper virus infection of dogs, visna virus infection of sheep, and murine coronavirus and Theiler's murine encephalomyelitis virus infection of mice (11,29,34-37). The presence of persistent virus infection in these animals results in demyelination with characteristic features similar to MS in humans.

Experimental Model

Experimental allergic encephalomyelitis (EAE) is an experimental model of MS. EAE can be induced in mice by injection of peptides from CNS proteins such as myelin basic protein, myelin proteolipid protein (PLP), or myelin oligodendrocyte glycoprotein (38). The type of EAE induced depends on the immunization protocol, the mouse strain, and the antigen used.

Our laboratory has developed a model, based on EAE, which provides evidence that multiple virus infections can induce autoimmunity (14). The initial infection primes the immune system, while a subsequent infection or other non-specific immunologic stimulus can trigger the disease. The mice used are 3- to 4-week-old (mimicking pre-puberty) female SJL/J mice, which are genetically susceptible to EAE. In our model, we use one of two different methods for the initial priming infection. The first is the injection of plasmid cDNA encoding ubiquitin fused to PLP. The second is infection with recombinant vaccinia virus encoding CNS proteins. These agents approximate what could occur during virus infection when virus proteins, having molecular mimicry with self-CNS proteins, are synthesized within the cytoplasm of infected cells. The subsequent infection or non-specific immunologic stimulus for the first priming method was either phosphate buffered saline (PBS) emulsified in complete Freund's adjuvant (CFA) or a recombinant vaccinia virus encoding β-galactosidase. PLP$_{139-151}$ emulsified in CFA was used as a positive control and PBS alone as a negative control. The subsequent non-specific immunologic challenge for the second priming method was CFA, either with or without whole *Bordetella pertussis* organisms.

Mice primed with the cDNA plasmid and triggered with CFA developed clinical and pathological signs of EAE, similar to that seen in the control group primed with cDNA plasmid and triggered with PLP$_{139-151}$ (positive control). Mice primed with the cDNA plasmid and triggered with the recombinant vaccinia virus encoding β-galactosidase developed pathological signs of EAE without any clinical signs. Also, mice primed with the recombinant vaccinia virus encoding CNS proteins and challenged with CFA plus *B. pertussis* developed pathological lesions of EAE; however, no clinical signs were evident. The presence of pathological lesions in the absence of clinical symptoms has also been observed in MS in humans. Through the use of magnetic resonance imaging, disease activity in the form of pathological lesions has been observed to be 5 to 10 times higher than the disease activity recognized by clinical evaluation (39-41).

These findings show how an initial early infection with a virus whose antigens display molecular mimicry to self-epitopes can prime for disease that occurs after a subsequent, non-specific immunostimulatory event. Encounters with microbes at a young age, therefore, can set the stage for the development of autoimmune disease later in life.

Conclusions and Speculations

If, as is seen in our model, an initial encounter with a microbe can prime for disease without causing overt CNS disease itself, and disease occurs after an encounter with some non-specific agent, then this could explain why no one specific virus has been demonstrated to cause MS. The initial priming event, as well as the triggering event, may be caused by a variety of microbes, which may explain to some extent the variability in the clinical course of MS seen in individual patients.

Acknowledgements

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Controversies in *Clostridium difficile* Testing

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Abstract

Recent reports of two nosocomial outbreaks of *Clostridium difficile*-associated disease caused by toxin A-deficient strains emphasize that these strains can cause disease. Laboratories using an assay that detects only toxin A as their primary diagnostic test risk misdiagnosis of cases or outbreaks in the institutions they serve. Repeat testing can account for a significant portion of a laboratory's *C. difficile* testing workload. Published data are available to support laboratory rules for rejection of repeat stool specimens within 7 days of an initial specimen. There are also substantial published data to support laboratory rejection of formed stools sent to the laboratory for *C. difficile* testing.

Introduction

Clostridium difficile is associated with 90 to 100% of cases of pseudomembranous colitis, 60 to 75% of antibiotic-associated colitis, and 11 to 33% of antibiotic-associated cases of diarrhea (1). Asymptomatic carriage is thought to be two- to five-fold more common than the disease itself. The

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organism produces two exotoxins: toxin A, which is thought to be responsible for most of the gastrointestinal symptoms seen, because it functions as an enterotoxin; and toxin B, which is a potent cytotoxin. A working definition of *C. difficile*-associated disease (CDAD) includes (i) diarrhea, defined as at least six watery stools over 36 h, three unformed stools in 24 h for 2 days, or eight unformed stools over 48 h; (ii) pseudomembranes seen at endoscopy of the lower gastrointestinal tract or detection of toxins or toxigenic organisms in stool; (iii) no other recog-

nized etiology for diarrhea; and (iv) a history of treatment with antimicrobial or antineoplastic agents within the previous 8 weeks or a response to specific therapy for CDAD (2). Many antimicrobial and antineoplastic agents have been demonstrated to induce CDAD, but the most common are the antimicrobial agents clindamycin, ampicillin, amoxicillin, and the cephalosporins (3).

Diagnosis of CDAD is important because the disease can be severe and it is a major nosocomial problem. A variety of methods are currently available for the laboratory diagnosis of CDAD,