

**SUSPECTED MECHANISMS INVOLVED IN MULTIPLE
SCLEROSIS AND PUTATIVE ROLE OF HEPATITIS B VACCINE
IN MULTIPLE SCLEROSIS**

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The Immunization Safety Review Committee's upcoming meeting examines the Hepatitis B vaccine and neurological disorders. As one of its data gathering activities, the committee asked Drs. Emmanuelle Waubant and Olaf Stuve, of the University of California at San Francisco, to write a paper addressing diagnostic issues and mechanistic issues related to the putative relationship between Hepatitis B vaccine and demyelinating disease. The committee welcomes comments on this material.

The committee wishes to note that this represents the views of Dr. Waubant and Dr. Stuve and does NOT necessarily reflect the conclusions the committee will draw when it deliberates at its March meeting. The committee further notes that this paper is one source of many that it will review in the course of its deliberations.

Comments on this material can be emailed to the committee at imsafety@nas.edu. It is most helpful if comments are received by March 7, 2002. **COMMENTS WILL BE PART OF THE PUBLIC ACCESS FILE FOR THIS PROJECT AND PERSONAL IDENTIFIERS WILL BE INCLUDED.**

For your information the agenda for the open scientific workshop to be held on March 11, 2002 in Washington, DC has been posted at www.iom.edu/imsafety. In addition, a list of materials sent to the committee to date will be posted later this week at www.iom.edu/imsafety.

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1. Current understanding of multiple sclerosis

a. General considerations

Multiple sclerosis (MS) is the most common chronic inflammatory demyelinating disease of the central nervous system (CNS) in humans. Approximately 300,000 (0.1%) individuals in the United States have been diagnosed with MS. Women are affected approximately twice as often as men. While the highest incidence is between ages 20 and 40 years, young children and older individuals have been diagnosed with this illness. MS is more frequent in Caucasians than in other ethnic groups. The prevalence of MS varies between 50 and 250 per 100,000 in high-risk areas such as the Scandinavian countries or the Northern United States, to less than 5 per 100,000 in Africa and Japan.

Clinically, MS may result over time in the accumulation of various neurological disabilities. Inflammatory demyelinating lesions in MS can occur throughout the CNS, accounting for the variety of signs and symptoms that may develop in individual patients. Common presenting symptoms include focal sensory deficits, focal weakness, a loss of vision, double vision, imbalance, and fatigue. Sexual impairment as well as urinary and bowel dysfunction may occur. Approximately 50% of patients with MS may display some degree of cognitive impairment and psychiatric symptoms.

Four disease patterns have been identified (Lublin and Reingold, 1996). More than 80% of patients with MS experience initially a relapsing-remitting (RR) course with

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clinical exacerbations of neurological symptoms, followed by recovery that may or may not be complete. Exacerbations last from one day to several weeks. During RRMS, accumulation of disability may occur due to incomplete recovery of relapses. Approximately 50% of patients with RRMS will experience a more progressive course of the disease after 10 years of RR course. This is called secondary progressive (SP) MS. During this SP course, patients' disability gradually worsens with or without superimposed exacerbations. Another 10-15% of the patients have primary progressive (PP) MS, a form associated with gradual progression of symptoms from onset without exacerbation or remission. A very small proportion of patients (1-5%) experience a course called progressive relapsing (PR) MS, which is progressive from onset with very few superimposed exacerbations during the entire course of the disease.

The severity of MS can vary widely from sub-clinical disease diagnosed upon autopsy in individuals who died from other causes, to hyperacute forms with death within the first few months after disease onset. Although 20% of patients with MS have a "benign" form of the disease, which is characterized by little accumulation of disability after 10 or more years of evolution, 50% of the patients develop a significant limitation to ambulate and require assistance after 10-15 years of MS evolution.

The clinical diagnosis is made after the occurrence of recurrent episodes of clinical exacerbations (dissemination in time) representing dysfunctions in different anatomic locations within the CNS (dissemination in space). Magnetic Resonance Imaging (MRI) is the most sensitive paraclinical diagnostic test to support MS diagnosis. T2- and fluid-attenuated inversion-recovery (FLAIR) weighted MRI can detect white

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matter lesions in 95% of the patients. Examination of cerebrospinal fluid (CSF) from MS patients often reveals a mild pleocytosis. An elevated IgG index (ratio of CSF to serum IgG corrected for albumin concentration in each compartment) indicates there is intrathecal IgG synthesis. Two or more oligoclonal IgG bands within the CSF are detected by electrophoresis in more than 80% of MS patients. Evoked potentials are sometimes helpful in identifying neurological dysfunctions that are not apparent clinically or on CNS imaging. Although highly sensitive, none of these tests are specific for the diagnosis of MS. In the recent past, diagnostic criteria have been utilized by clinical neurologists in order to facilitate the reproducible diagnosis of this disease (Poser et al., 1983). Recently, an international panel of MS experts developed new diagnostic criteria that account for the increasing contribution of MRI findings in the diagnosis of MS (McDonald et al., 2001).

b. Definition of multiple sclerosis onset

The onset of MS is currently defined by the occurrence of neurological symptoms caused by CNS dysfunction. As for the diagnosis of MS dissemination in time and dissemination in space are required, the diagnosis is often established only after the second clinical attack, and the clinical disease onset defined retrospectively as the first clinical attack. In the majority of the cases, typical findings on MRI may contribute to the diagnosis of MS at the time the patient experience the initial symptoms. Indeed, the McDonald diagnostic criteria have now included the MRI to take in account

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dissemination in space (3 of 4 of the following criteria: (1) One gadolinium enhancing lesion or 9 T2 bright lesions if there is no enhancement, (2) at least one infratentorial lesion, (3) at least one juxtacortical lesion, (4) at least three periventricular lesions) and dissemination in time (a new T2 or a Gadolinium-enhancing lesion on a repeat MRI provide sufficient evidence for dissemination in time)(McDonald et al., 2001).

The fact that a significant proportion of patients have already old MRI findings at the time they present their initial symptoms suggests that biological manifestations of the disease have started before clinical symptoms occur.

c. Definition of multiple sclerosis relapses

Disease relapses are defined by the clinical onset of new, recurrent or worsening neurological symptom related to CNS dysfunction that last for 24 or more hours in the absence of fever or infection. The McDonald diagnostic criteria define attacks as either subjective report or objective observation of CNS symptoms lasting at least 24 hours (McDonald et al., 2001). This assumes that there is a clinical assessment to rule out a “pseudo-exacerbation” that could be caused by fever or infection. However, some subtle symptoms such as sensory symptoms without clinical findings or cognitive impairment may not be acknowledged as confirmed exacerbations.

Interestingly, disease activity on neuroimaging studies does not always correlate with clinical symptoms and disability. Only lesions in physiologically relevant areas and

of sufficient size will give rise to neurological symptoms. In fact, for every new clinical attack, five to ten new plaques may be detected on serial brain MRI scans.

d. Etiology of multiple sclerosis

The cause of MS remains elusive, but disease susceptibility involves genetic and environmental factors.

Genetic susceptibility: 15 to 20% of patients with MS do have another member in their family affected by MS. The risk of developing MS is 10-20 times higher for first-degree relatives of affected individuals than in the general population. However, the risk to develop MS in individuals whose parents or siblings have MS remains small (1-3% in a life time). The concordance rate among dizygotic (fraternal) twins and other siblings is 2-5%, while it is 30-35% in monozygotic (identical) twins. Genetic studies of MS families with more than one member affected indicate that the major histocompatibility complex (MHC) class II region on chromosome 6p21 is the most consistently identified susceptibility locus. The strongest association is with HLA-DR2 (DRB*1501, DQB*0602)(Oksenberg et al., 2001). However, these investigations suggest that as many as 15-20 other loci may contribute to MS susceptibility, most of which still have to be further investigated. Some genes may participate to the susceptibility to develop the disease when others may modify the clinical expression of the disease or the response to disease modifying treatments. Interestingly, there are regional differences with regard to clinical disease patterns. For example, in Asia, although the overall incidence of MS is

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lower than in USA, the clinical presentation includes more typically bilateral visual loss and weakness of the extremities. Unlike western form of MS, which is associated with HLA-DR2 (DRB*1501), Asian MS appears to have a different genetic predisposition.

Environmental factors: Several epidemiological studies have suggested that individuals who migrate from regions with high disease prevalence to regions of low disease prevalence, or vice versa, after age 15 carry their native risk for contracting MS (Kurtzke et al., 1970; Alter et al., 1966). This suggests that exposure to an environmental factor, possibly an infectious agent, during childhood is critical for the development of MS. Reports of localized clusters, defined areas of unexpected high prevalence of MS, have further suggested that a transmissible agent may contribute to this illness.

e. Putative mechanisms involved in the development of multiple sclerosis

Macroscopically, MS lesions, i.e. demyelinated plaques (the French word for “scar”), are typically confined to CNS white matter and are found most frequently in periventricular areas of the brain, but also in the optic nerves, the brain stem, and the spinal cord. Located primarily in the perivascular zones within these structures, the size of the plaques can vary from a few millimeters to several centimeters. The fact that lesions are centered by venules suggests that extravasating systemic immunocompetent cells participate in plaque formation. Typically, acute and chronic lesions are found at the same time, and are thought to account for the appearance of clinical signs and symptoms at separate times. In addition to demyelination, it has been demonstrated that

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axonal loss occurs both in acute and in chronic plaques (Trapp et al., 1998). Demyelination and axonal loss eventually result in CNS atrophy, and contribute to the accumulation of neurological disability.

Microscopically, infiltration of the CNS by inflammatory cells such as CD4+ and CD8+ T cells, B cells, plasma cells and macrophages is usually observed in early stages of MS lesion development. In chronic lesions, reactive astrocytes and phagocytic macrophages are the predominant cell types. Typically, the number of oligodendrocytes, the CNS myelin forming cells, is decreased in older MS lesions compared to early lesions. Demyelination and axonal damage sometimes occur in the absence of inflammatory cell infiltrates, suggesting that CNS immunocompetent cells such as microglia or astrocytes could be a source of deleterious mediators.

It is currently thought that activated myelin-reactive CD4+ Th1 T cells have a central role in the pathogenesis of MS. Myelin basic protein (MBP), proteolipoprotein (PLP), and myelin oligodendrocyte glycoprotein (MOG) are the most commonly investigated candidate CNS autoantigens. The frequency of activated, CNS-antigen-specific CD4+ T cells is increased in MS patients. There is also a disproportionate number of activated CD4+ T cells within MS lesions, especially at the leading (active) edges. However, it is unclear if these T cells are myelin antigen-specific. Several immunoregulatory defects occur in MS. MS is associated with relative deficiency in activity of regulatory (suppressor) Th2 cells that secrete IL-4 and IL-10. Not surprisingly, myelin reactive CD4+ T cells from MS patients secrete increased amounts of the Th1 cytokines interferon- γ and IL-2 (Figure 1). Interferon- γ is a strong inducer of

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MHC class II and costimulatory (B7-1 and B7-2) molecules on nonprofessional antigen presenting cells (APC), including CNS resident APCs like astrocytes and microglia, which are thought to present myelin antigens to pathogenic T cells (Figure 1). Myelin-specific CD4⁺ T cells in individuals with MS also secrete other important pro-inflammatory cytokines, such as TNF, and chemokines. CD4⁺ T cells from MS patients express CD40 ligand, which binds CD40, a potent costimulatory molecule expressed on some APC. This ligand-receptor interaction causes APC to produce IL-12, a cytokine that induces secretion of interferon- γ and promotes Th1 differentiation.

Whether T cells have a primary role in the initial events leading to myelin destruction is unknown. It is also unclear whether the inciting events occur within or outside the CNS. CD4⁺ T cells recognize antigen in association with MHC class II molecules. However, the normal CNS is almost devoid of MHC class II molecules. Furthermore, the normal brain contains an intact blood brain barrier (BBB), which is composed of cerebrovascular endothelial cells connected by tight junctions. Activated, but not resting, lymphocytes can penetrate the BBB and enter the CNS parenchyma.

Humeral immune responses have also been implicated in the pathogenesis of MS, and oligoclonal bands in the cerebrospinal fluid of MS patients have long been thought to eventually provide diagnostic clues to the disease-causing antigen. Plasma cells within demyelinating lesions are thought to be responsible for increased intrathecal levels of IgG and presence of oligoclonal IgG. Potentially, antibodies may participate to CNS damage in MS through different mechanisms such as activation of the complement cascade and promotion of phagocytosis by phagocytic macrophages. Oligoclonal bands are not

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pathognomonic for MS, but have also been detected in numerous infectious diseases of the CNS. Interestingly, in subacute sclerosing panencephalitis (SSPE, a complication of live measles infection), human T-lymphotropic virus (HTLV)-1, mumps meningitis, neurosyphilis, progressive multifocal leukoencephalitis (PML; an infection with a papova virus), and cryptococcal meningitis, intrathecal oligoclonal bands represent antibodies directed against the causative agent.

f. Animal models

Substantial evidence for the role of myelin-specific T cells in CNS demyelinating diseases has been derived from investigations of experimental autoimmune ("allergic") encephalomyelitis (EAE), the archetypal model for MS (Zamvil and Steinman, 1990). Activated CD4⁺ Th1 cells that recognize one of the candidate myelin antigens mediate EAE, causing relapsing paralysis and CNS inflammation and demyelination. More recently, the pathogenic role of CD8⁺ T cells in EAE was better defined and supports a major role for them in CNS autoimmune disease. One study demonstrated that CD8⁺ T cell clones specific for a MHC class I restricted–fragment of MBP, produce severe EAE (Huseby et al., 2001). In this particular model, pathological sections showed areas of demyelination and perivascular infiltrates of immune-competent cells in the CNS that are more resembling of MS lesions than other EAE models. In another study, CD8⁺ T cells reactive to MOG peptide 35–55 resulted in similar clinical and pathological findings (Sun et al., 2001).

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Important concepts regarding the pathogenesis of MS have emerged from studies in EAE. In one model of "molecular mimicry", a microbial antigenic determinant cross-reacts with a determinant of a self-protein, an interaction that eventually leads to autoimmune destruction of host tissue (Benoist and Mathis, 2001) (Figure 2). The microbial antigenic determinant has to be a linear peptide of about 8–15 amino acids to be recognized by T cells, but different enough from the self-determinant to be recognized as foreign by the host's immune system. Although no single virus has proven to cause MS, several different viruses, including HTLV-1 and other retroviruses, herpes virus-6, and Epstein-Barr virus have been implicated in MS pathogenesis. In support of the role of molecular mimicry, myelin protein-specific CD4+ T cell clones from some MS patients react with proteins derived from some of these viruses, and immunization with specific viral peptides that share homology with myelin proteins can induce EAE. "Bystander activation" may also play a role in the cascade of events leading to demyelination. Bystander activation pertains to host tissue destruction that subsequently results in the release of large quantities of normally sequestered host proteins as a result of a microbial infection. These proteins could then be presented to T cells locally or in the draining lymph nodes.

Superantigens, natural proteins produced by certain viruses and bacteria, are other potential stimuli that may activate T cells to cause demyelination (Figure 3). Several mechanisms for superantigens have been suggested (Brocke et al., 1994). First, superantigens may directly activate auto-reactive T cells through their T cell receptor, regardless of antigen specificity. These T cells could then distribute to non-lymphatic

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tissue and mediate inflammation through the release of inflammatory mediators, including pro-inflammatory cytokines and chemokines. Second, superantigens may activate a humeral immune response in two different ways. They can crosslink the T cell receptor with the MHC class II molecules on B cells resulting in antibody production and formation of immune complexes that can participate to demyelination. Superantigens may also activate B cells through their immunoglobulin receptor itself. Third, superantigens may lead to the release of inflammatory mediators such as pro-inflammatory cytokines and superoxides through the activation of macrophages. The activation of macrophages by superantigens may have further implications in autoimmune processes, as possible alterations in auto-antigen processing and presentation, may lead to activation of auto-reactive T lymphocytes. The ability of staphylococcal enterotoxin B, a superantigen that binds the subfamily of T cell receptor V β chains used by some encephalitogenic T cells, to exacerbate the clinical course of EAE was recently demonstrated.

It has been observed in EAE that the initial onset of clinical disease, following immunization with a major (dominant) determinant of a CNS antigens, is often followed by disease relapses due to recruitment of CD4⁺ T cells that recognize secondary (subdominant or cryptic) determinants of the same autoantigen or a determinant(s) of another encephalitogenic autoantigens (Vanderlugt and Miller, 1996). This observation has led to development of the concepts of intramolecular and intermolecular determinant spreading (also: “Repertoire broadening”, or “epitope spreading”), respectively. Evidence indicates that determinant spreading is important in the development of relapses

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in EAE and may have similar importance in the development and progression of MS and other diseases.

More recently, investigators have examined the role of B cells in these conditions. B-less (μ -knock-out) mice are susceptible to clinical EAE and develop CNS demyelinating lesions, indicating that B cells are not obligatory for induction of CNS demyelinating disease (Lyons et al., 1999). Nevertheless, in recent studies, autoantibodies specific for MOG were detected in acute MS lesions (Genain et al., 1999). Furthermore, transfer of MOG-specific autoantibodies led to demyelination and clinical disease in certain EAE models. Thus, there is now considerable interest in further characterizing the role of myelin-specific antibodies in MS.

2. Mechanisms underlying “initiation” versus “relapse” of multiple sclerosis

a. Mechanisms that possibly trigger multiple sclerosis onset

Genetic factors and environmental factors are suspected to participate in the development of MS. All the putative mechanisms described in the previous sections may be responsible alone or in combination for initiating the disease either acutely, or following an incubation period of variable length.

b. Mechanisms that possibly trigger multiple sclerosis relapses

Various mechanisms that trigger relapses of clinical disease in EAE have been described (see above). It remains unclear, if molecular mimicry, superantigens, or epitope spreading play a role in MS relapses. However, there are situations, during which the frequency of clinical exacerbations is increased in patients already diagnosed with MS. First, the three-month period following delivery is a period during which the risk of developing a clinical relapse in MS patients is multiplied twofold (Confavreux et al., 1998). Consequently, it is suspected that hormonal changes per se or immunological changes related to the hormonal changes participate to the increased risk. Second, several agents increased the relapse rate of MS during clinical trials, including interferon (IFN) α (Panitch et al., 1987), and Lenercept, a recombinant TNF receptor p55 immunoglobulin fusion protein (The Lenercept MS Group 1999). Interestingly, the

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administration of an MBP peptide (aa 83-99) designed as an altered peptide ligand (APL) was followed by disease exacerbations in several patients (Bielekova et al., 2000). In two of these individuals, the clinical relapses could be linked to the APL by immunological studies, thus suggesting the encephalitogenic potential of this particular MBP peptide in a subgroup of patients (Bielekova et al., 2000).

Since the original description of MS by Charcot, a number of factors have been implicated in disease relapses, including physical trauma, psychological stress, and infections. However, based on a report recently published by the Therapeutic and Technology Assessment Subcommittee of the American Academy of Neurology, a panel of experts that reviewed all evidence currently available, an association between trauma and MS exacerbations can be excluded (Goodin et al., 1999). A relationship between antecedent psychological stress and either MS onset or MS exacerbation was considered possible (Goodin et al., 1999). Another review of published evidence concluded that while there is conflicting evidence on the risk of common infectious diseases in the MS population compared to healthy individuals, there is definite evidence for an increased risk of MS exacerbations during an infectious episode (Rutschmann et al, in press).

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3. CNS disorders that mimic multiple sclerosis

a. Differential diagnosis of multiple sclerosis

A number of infectious, inflammatory, and autoimmune conditions, including neurosarcoidosis, neurosyphilis, Lyme Disease, systemic lupus erythematosus, CNS vasculitis, Behcet's disease, and Sjögren's syndrome, can sometimes produce signs, symptoms, spinal fluid and neuroimaging abnormalities similar to that in MS. Human T lymphotropic virus (HTLV)-1-associated myelopathy or spinocerebellar degenerative disorders are considered when patients present with slowly progressive weakness of the lower extremities. Certain metabolic disorders, including vitamin B12 deficiency and hypothyroidism, are also considered in the initial diagnostic evaluation. A family history and the use of appropriate diagnostic tests allow clinicians to rule out most diseases that mimic MS (Stüve and Zamvil, 2001).

Some neurologists consider acute disseminated encephalomyelitis (ADEM), Marburg type of acute MS, Balo's concentric sclerosis, and neuromyelitis optica as variants of MS. For the purpose of this work, only ADEM will be discussed.

b. Acute disseminated encephalomyelitis

Acute disseminated encephalomyelitis (ADEM) is generally considered to be a monophasic demyelinating disease of the CNS (Stüve and Zamvil, 1999). Interestingly, an incidence of 0.1% of ADEM was reported after the introduction of the first vaccine

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against the rabies virus in humans. This particular vaccine was manufactured from rabbit spinal cord homogenate containing attenuated rabies virus, and the immunization thus initiated the human equivalent of EAE. ADEM most often follows an infection. However, it is sometimes reported after various vaccinations despite the fact that vaccines do not contain CNS tissue anymore. Sometimes a history of a preceding infection is difficult to obtain. Neurologic symptoms can begin during (parainfectious) or shortly after (postinfectious) the acute viral illness. Following a vaccination, clinical symptoms usually occur after one to three weeks. However, the Viral Hepatitis Prevention Board, a World Health Organization (WHO) Collaborating Center for the prevention of viral hepatitis, cited a period of up to three months after vaccination as an acceptable time frame for the diagnosis of ADEM related to the vaccine. Despite the fact that ADEM is uncommon, it is important for several reasons. First, vaccinations are increasingly widespread. Second, ADEM can result in permanent and severe neurological disability.

A number of viral pathogens have been associated with ADEM, including the Measles-, Rubella-, and Varicella zoster viruses and less commonly, Influenza-, Mumps-, Coxsackie B-, Epstein-Barr-, Herpes simplex (HSV), Human immunodeficiency (HIV), and Human herpes-6 (HHV-6) viruses. The incidence of ADEM after measles is approximately 1 out of 1000 infections, whereas after varicella and rubella it is less than 1:10,000 and 1:20,000 respectively. ADEM subsequent to infections with *Mycoplasma pneumoniae* and *Legionella cincinnatiensis* have also been reported.

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The incidence of post-immunization ADEM is 1-2 per 10⁶ for live measles vaccine immunizations, i.e. significantly lower than that for post-measles. It is most commonly associated with measles, mumps, and rubella vaccinations. More recently, possibly due to their later introduction, case reports and case series have reported post-immunization ADEM following inoculation with two recombinant hepatitis B vaccines containing hepatitis B surface antigen (HbsAg).

ADEM usually affects infants and young children, although infrequently it has been reported in middle aged and elderly individuals. Three recent case series, two in pediatric, one in adult patients, suggest that there may be differences with regard to clinical presentations according to age of onset (Hynson et al., 2001; Schwarz et al., 2001; Dale et al., 2000). Typically, in both post-infectious and post-immunization ADEM, patients initially develop fever and nonspecific respiratory illness. Common clinical features of ADEM include meningismus, ataxia, weakness, and spasticity. In pediatric patients, headaches, seizures, impaired conscious state and sometimes coma associated with respiratory distress seems to be more frequent, whereas adult patients often display milder neurological symptoms and less often fever and infectious symptoms. After a period of stabilization, patients frequently improve. Transverse myelitis may represent a variant of ADEM restricted to the spinal cord and also occurs commonly after viral or bacterial infections.

The diagnosis of ADEM is strongly suggested by a close temporal relationship between the onset of the neurologic symptoms and a viral exanthem or immunization. This diagnosis is supported by high white blood cell counts, elevated sedimentation rate

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and extensive subcortical white matter disease on brain MRI scans. The CSF often shows a mild lymphocytic pleocytosis and elevated proteins. Elevated CSF IgG and the presence of oligoclonal bands on electrophoresis are frequently detected in adult patients suspected of ADEM, but are less frequent in children. As in other conditions causing acute injury to CNS myelin, increased cellular immune responses to myelin basic protein have also been demonstrated.

As previously mentioned, ADEM is by definition a monophasic disease. However, there have been some case reports and case series in which a significant proportion of children and young adults have developed subsequent neurological relapses that occurred after a period of complete or partial remission. Relapses may be identical or distinct from presenting symptoms. When relapses occur within the next few weeks (up to 2 months after onset of ADEM), e.g. during or just after steroid taper, they should be considered a continuation of the initial symptoms. Many pediatric neurologists refer to this disease entity as “relapsing disseminated encephalomyelitis” (RDEM)(Stüve and Zamvil, 1999) or “multiphasic disseminated encephalitis” (MDEM)(Dale et al., 2000). However, in other cases, the recurrence occurs years after the initial symptoms and discontinuation of steroids (Hynson et al., 2001). These cases would then meet criteria for RRMS. There is unfortunately no consensus to differentiate ADEM recurrences from MS relapses. Further, studies with conventional MRI and CSF have failed to establish specific criteria to differentiate ADEM, MS, or any of the other disseminated CNS white matter diseases mentioned above because of significant overlap. Consequently, the

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diagnosis of ADEM can only be confirmed retrospectively when no exacerbation has occurred in the years following the initial event.

Different animal models have suggested that both an infectious mechanism and a secondary autoimmune response contribute to CNS demyelination during the course of ADEM. Infection of susceptible mice with Theiler's murine encephalomyelitis virus (TMEV) causes acute encephalitis and CNS demyelination associated with activation of CD4+ and CD8+ T cells (Begolka et al., 2001). A fulminant monophasic EAE and variable degrees of CNS demyelination occur following immunization with myelin extract or purified myelin antigens in complete Freund's adjuvant. As for MS, myelin-specific T cells are thought to have a central role in the pathogenesis of ADEM. Humoral immune responses to CNS autoantigens (e.g. gangliosides) may also be involved.

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4. Scientific legitimacy of the hypothesis that hepatitis B vaccine could cause central demyelinating disease

a. Biological plausibility

In 1982, the first vaccine consisting of spherical forms of HbsAg from the plasma of healthy HbsAg carriers was introduced for active immunization. Five years later, this vaccine was replaced by a genetically engineered vaccine derived from recombinant yeast. Two recombinant vaccines are currently approved by the Food and Drug administration (FDA) for use in the United States. Both preparations consist of non-glycosylated HbsAg and are indistinguishable from natural HbsAg with regard to antigenicity.

At least one antigenic determinant on human HbsAg has been identified (Pride et al., 1992). A linear 15 amino acid peptide generated a humeral anti-HBsAg-specific immune response when injected into mice.

A study in 1985 reported that hepatitis B (HB) virus polymerase (VP) shares six consecutive amino acids (75%) with an encephalitogenic site of rabbit MBP (Fujinami and Oldstone, 1985). Peripheral blood leukocytes of rabbits immunized with HBVP proliferated in vitro to the viral peptide and intact MBP. Furthermore, animals injected with an eight- or ten-amino acid peptide from HBVP showed an antibody response against HBVP and native MBP. However, while the authors of this study were able to demonstrate inflammatory infiltrates in the CNS of animals immunized with HBVP, they

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were unable to elicit clinical EAE (Fujinami and Oldstone, 1985). The authors concluded that viral infections may trigger the production of antibodies and mononuclear cells that cross-react with self proteins by molecular mimicry.

Despite these interesting observations, there is still no conclusive scientific evidence that molecular mimicry between HB vaccine proteins and CNS antigens exists and plays a role in the pathogenesis of MS. In fact, there is no significant homology between the amino acid sequences of HBsAg, the main component of the HB vaccine, and MOG, MBP and PLP. Thus, it is unlikely that a T cell mediated immune response against these CNS autoantigens would be triggered by HB vaccine on the basis of molecular mimicry. A humeral autoimmune response may still be conceivable, as antibodies recognize both conformational and linear epitopes. Although several studies reported the specificity of intrathecal oligoclonal bands against specific pathogens in other CNS diseases, no antibody against HB virus, or more specifically against HbsAg has been identified in MS.

The fact that an infection with the natural hepatitis B virus has not been proven to cause MS or worsen clinical disease activity may further argue against molecular mimicry between HbsAg as the principal component of recombinant hepatitis B vaccine and CNS antigens. Also, there is currently no conclusive evidence that viral or bacterial superantigens cause MS onset or MS exacerbations.

b. Clinical plausibility

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As mentioned earlier, the onset of MS is currently defined by the appearance of clinical symptoms. However, many patients have evidence of old CNS lesions on brain and/or spinal cord MRI at the time they present with their initial symptoms (Ormerod et al., 1986). This indicates that biological manifestations of the disease started before the onset of clinical disease, and that the use of brain MRI scans may provide a more sensitive measure of disease activity. Relying solely on clinical outcome measure could thus limit the ability of clinical studies to detect a role for HB vaccine in the development of MS. To study the possibility of subclinical CNS inflammation after HB vaccine in a prospective manner, patients could be randomized to receive either HB vaccine or placebo. Neurological evaluation and MRI scans of the brain could be obtained prior to immunization, and study participants could be monitored clinically and with monthly brain MRI scans. One study that looked at the effect of influenza vaccine on MS activity showed no increase in MRI activity 15 and 45 days after immunization (Salvetti et al., 1997).

The putative temporal relationship between HB vaccine and the development of MS is unknown. It is currently believed, based on anecdotal clinical observations, that an increased risk would be apparent shortly after an immunization. It is conceivable however, that immunization could increase the long-term risk of developing MS. This unknown time frame limits our ability to detect a putative relationship and may call for study designs addressing particularly this issue. Essentially, there have been two different types of studies examining a possible relationship between HB vaccine and MS: (1) anecdotal reports, and (2) studies specifically designed to investigate this relationship.

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While anecdotal reports suggest a relationship between hepatitis B vaccine and MS without showing causality, the second type of studies rule out this relationship.

In the first type, information regarding the majority of clinical cases is derived retrospectively. This includes information regarding time and characteristics of initial symptoms, and objective neurological findings may not be mentioned. Some of these anecdotal cases were prospectively followed from the onset of neurological symptoms at the same institution. Consequently, these reports are less likely to suffer from recall biases. It is also notable that some of these cases were published during period of heightened public alertness regarding possible health hazards resulting from HB vaccine.

The second type of studies, usually large multicenter studies that have evaluated the relationship between HB vaccine and MS are either fully prospective or include retrospective and prospective information. In one study, data was derived from a large national follow-up effort (Ascherio et al., 2001). Another large case cross-over study was conducted to determine the role of various vaccines on the risk of developing subsequent exacerbation in patients already diagnosed with MS (Zipp et al., 1999). Patients with MS-like symptoms that did not meet stringent criteria for definite MS according to Poser criteria were excluded from these two studies (Zipp et al., 1999; Ascherio et al., 2001). Unfortunately, in these studies patients with a history of an isolated neurological event and therefore at high risk to develop MS were excluded (Zipp et al., 1999; Ascherio et al., 2001). The incidence of MS in this cohort following HB vaccine may have been underestimated. The study that looked at MS exacerbations after HB vaccine required a one-year relapse-free interval as entry criteria (Confavreux et al., 2001). Consequently,

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patients with frequent exacerbations were excluded, and it is therefore problematic to generalize the results.

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5. Central nervous system demyelinating diseases in children

a. Multiple sclerosis and other central nervous system demyelinating diseases in children

MS exists in children, although much less frequent than in adults (Duquette et al., 1987). The incidence of MS before age 10 years is less than 3/1000. Pediatric cases of MS constitute 0.3 to 2% of all MS cases. Children as young as 2 years of age have been diagnosed with MS. It is perhaps noteworthy that monophasic demyelinating diseases such as ADEM and isolated optic neuritis (ON) are more frequent in children than in adults and that ON and ADEM are more frequently preceded by infection in pediatric cases (Hynson et al., 2001).

Some inflammatory CNS demyelinating diseases, including sarcoidosis have comparable incidences in children and in adults, whereas diseases such as Sjögren's syndrome or systemic lupus erythematosus are less common during childhood.

b. Functional differences between infant and adult immune response

Neonates are capable of mounting a protective immune response to vaccines within hours of birth. Young infants are fully capable of generating protective humoral and cellular immune response to multiple vaccines simultaneously. Approximately 90% of infants develop active protective immune responses to the primary series of vaccines

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such as HB vaccine given between 2 and 6 months of age (Offit et al., 2002). To circumvent the infant's inability to mount T-cell-independent B-cell responses, polysaccharide vaccines are linked to proteins that engage the infant's T-cells.

c. Differences between the mature and immature central nervous system

The structural maturation of the human CNS continues during childhood and adolescence. A recent study, investigating age-related increases in the cerebral white matter density utilizing structural MRI, demonstrated a temporal-spatial distribution of CNS myelin in corticospinal tracts and frontotemporal pathways (Paus et al., 1999). The molecular and cellular events underlying oligodendrocyte development and myelin synthesis of the CNS are not completely understood. During myelination, the synthesis of two major myelin-specific structural proteins, MBP and PLP, precedes the expression of MOG. It is therefore believed that MOG may be an integral factor in myelin sheath completion and maintenance. While different isoforms of some myelin proteins exist in humans, the biochemical composition of the major CNS myelin components remains very similar throughout all stages of postnatal development.

These quantitative and qualitative differences between immature and mature CNS tissue may account for some of the difference between MS prevalence between children and adults.

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d. Risk to develop central nervous system demyelinating diseases in children after hepatitis B vaccination

There is no evidence that vaccines in children cause more or less frequent CNS demyelination than in adults. One study showed that the incidence of demyelinating diseases was not increased after HB vaccine in the general population as well as in children under age 14 years (Zipp et al., 1999). Another report concluded that HB vaccine in children age 11-12 years did not increase the risk to develop MS in adolescence or ADEM (Sadovnick and Scheifele, 2000). Finally, the information derived from studies in adults regarding the lack of relationship between HB vaccine and MS onset and exacerbations is difficult to extrapolate to children. Since MS is very rare in children, it is difficult to design a study looking at changes in the risk to develop a relapse of MS following HB vaccine in this population.

6. Peripheral demyelinating diseases associated with vaccines.**a. Acute inflammatory demyelinating polyneuropathy: Guillain Barre syndrome**

Guillain-Barre syndrome (GBS) is the most common acquired peripheral demyelinating disease in humans. It often occurs several days or weeks after an infectious event. The majority of patients reporting a recent infection had a diarrheal illness preceding the onset of their symptoms, (Campylobacter jejuni infections represent up to 10 to 30 percent of all cases) or a viral upper respiratory infection. Viral pathogens that have been associated with GBS include Human immunodeficiency virus, Epstein-Barr virus and cytomegalovirus. There is also a small but definable increased risk of developing GBS after immunization, which became most apparent after the 1976 swine-flu vaccine program. Up to 5 percent of cases occur within 1 to 4 weeks of a surgical procedure. In the animal model of GBS, experimental allergic neuritis (EAN), similar clinical signs and laboratory features can be evoked by immunization with components of peripheral nerve such as galactocerebrosides, peripheral myelin P2 basic protein, peripheral nerve myelin, or whole peripheral nerve.

Lesions of the peripheral and cranial nerves are characterized histologically by infiltration with mononuclear cells and areas of focal distal and proximal segmental inflammation and demyelination. The presence of T cells, B cells, and macrophages around peripheral nerve lesions suggests a final effector pathway of cellular and humoral

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immune responses in myelin destruction. After a prolonged disease course, there may also be evidence of axonal loss and wallerian degeneration. In patients with evidence of *Campylobacter jejuni* infection, GBS is histologically characterized by axonal degeneration. In this particular variant, demyelination is either partial or completely absent.

The characteristic clinical feature of GBS is an acute, rapidly progressive, ascending, and symmetric weakness with loss of deep tendon reflexes and possible tingling (paraesthesias) in their feet and hands and muscle aches (myalgia). Facial, oculomotor, oropharyngeal, and respiratory muscles may also be involved, and some patients may require respiratory support for some time. The severity of clinical deficits typically peaks within the first two weeks of onset, but some will progress for 3 –4 weeks. Most patients will improve and return to normal function within 6-9 months. However, relapses and a prolonged disease course with residual neurological deficits have been reported.

Increased IgG and IgA antibody titers to GM1 ganglioside can be found in the axonal variant of GBS whereas antibodies to GQ1b are associated with the Miller-Fisher variant. Evidence of *Campylobacter* infection in almost a third of GBS patients may suggest a common antigen shared by this or other infectious agents (or vaccines) and a myelin epitope on the peripheral nerve, triggering an autoimmune response.

b. Potential differences between central nervous system and the peripheral nervous system demyelination

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Central and peripheral myelin is synthesized by oligodendrocytes and Schwann cells, respectively. The external cell membrane of each Schwann cell surrounds a single axon whereas oligodendrocytes typically ensheath several axonal processes. The expression of myelin genes by Schwann cells in the PNS is regulated by contact between the axon and the myelinating Schwann cell. In contrast, the expression of myelin genes by oligodendrocytes in the CNS appears to depend on the presence of astrocytes. Both, CNS and PNS myelin contains MBP and a distinct protein termed myelin-associated glycoprotein (MAG). More than half of the total protein in central myelin is PLP. The major protein in mature peripheral myelin is myelin protein zero (P₀). Consequently, either CNS or PNS antigens are used for induction of clinical disease in EAE and EAN, respectively.

Remyelination is more effective in the PNS than in the CNS likely for reasons related to the respective tissue environment (e.g. growth factors and other mediators). Clinically, GBS is considered a monophasic disease as opposed to MS, and its relationship with a previous infectious episode or vaccinations is better established than in MS.

7. Conclusions

Because MS is a very heterogeneous disorder with multifactorial pathogenesis, to date it has been impossible to test individuals for disease susceptibility on the basis of specific genes, gene products, or environmental factors. While a positive association between HB vaccine and MS remains a theoretical consideration, the best currently available biological and clinical evidence argue strongly against it.

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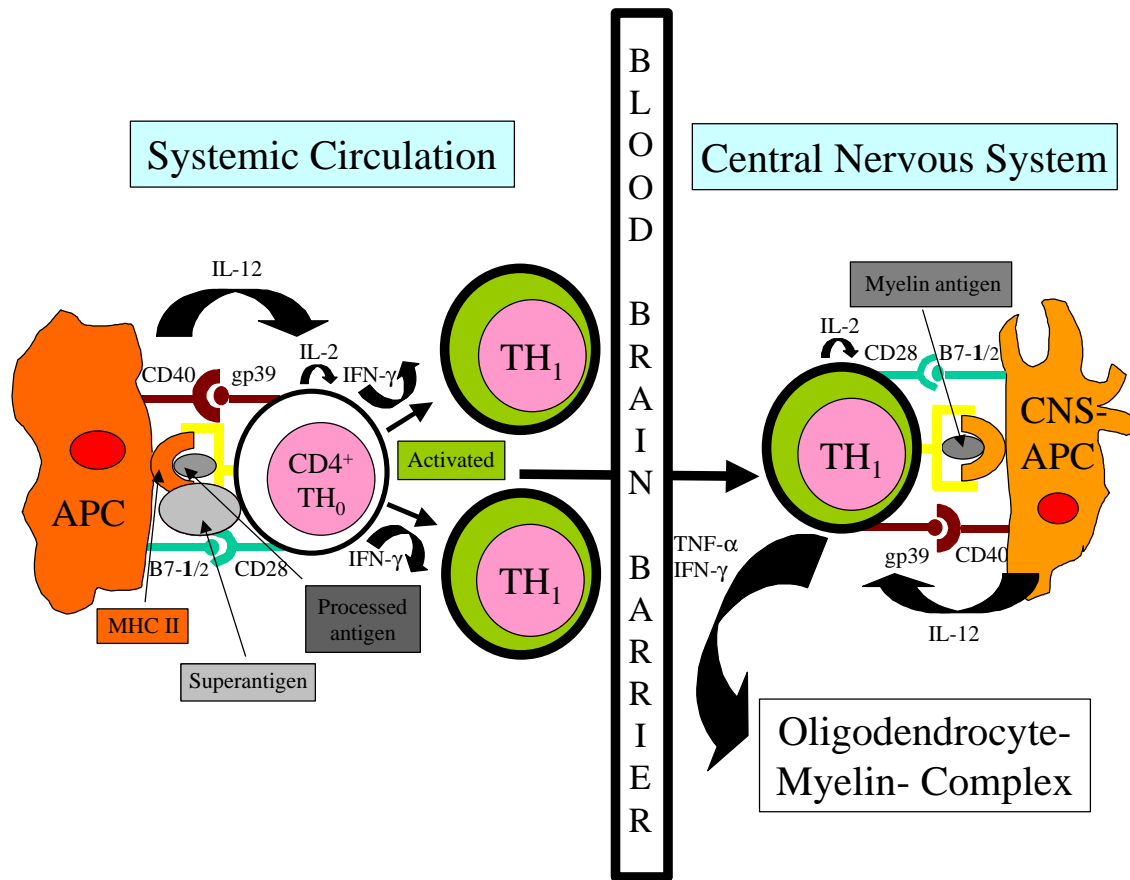


Figure 1: In experimental autoimmune encephalomyelitis (EAE), systemic infectious pathogens can initiate a $CD4^+$ cell mediated autoimmune response against central nervous system (CNS) antigen (Ag). The microorganism shares structural similarity or amino acid sequence homology with self Ag in the CNS (“molecular mimicry”). First, the Ag is presented to $CD4^+$ T cells by antigen presenting cells (APC). Peripheral myelin-reactive $CD4^+$ T cells can also be activated by superantigens, molecules that bind to MHC class II and the V_β domains of the T cell receptor. After activation and proliferation, $CD4^+$ cells differentiate into a cell type known as TH_0 cells, which have the potential to become inflammatory TH_1 T cells. TH_1 cells can be induced to secrete inflammatory cytokines upon engaging to myelin Ag, which may be presented by systemic or CNS-APC.

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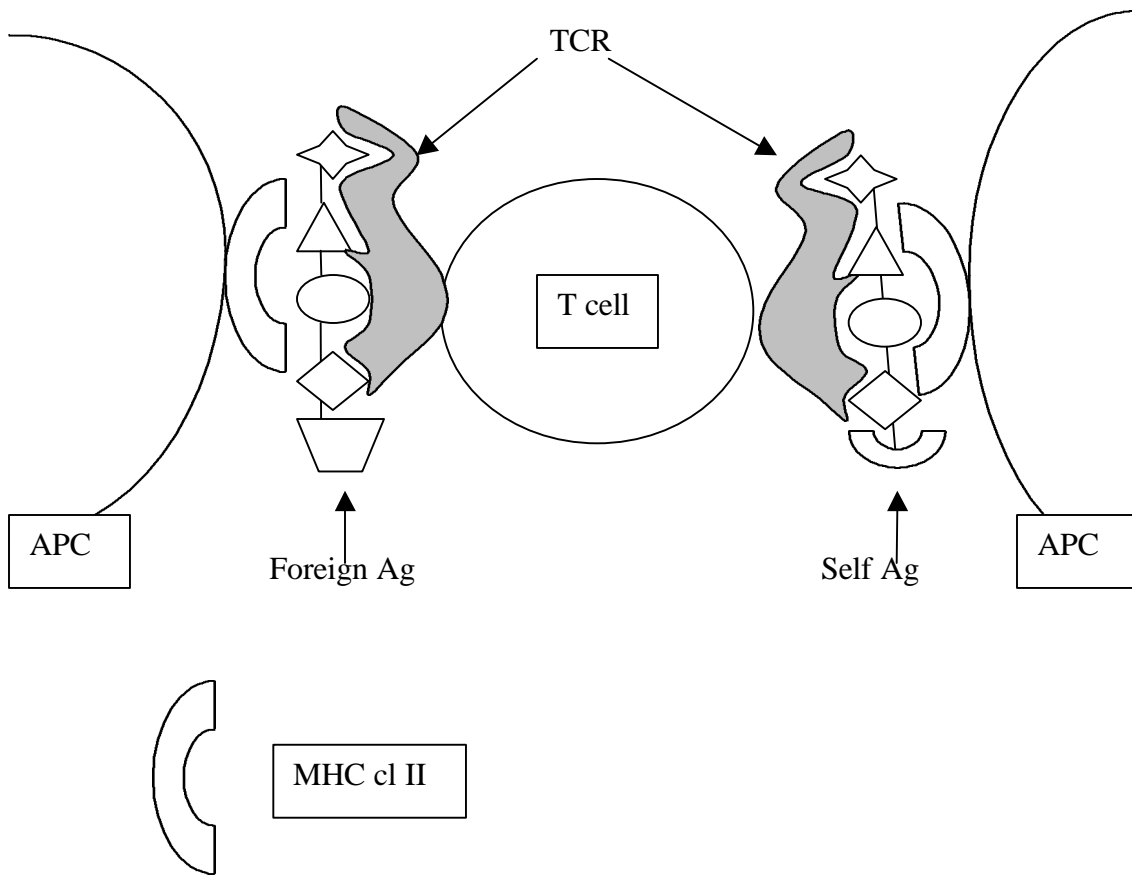


Figure 2. Molecular mimicry

The TCR can recognize an antigen that has a structure close to its specific antigen.

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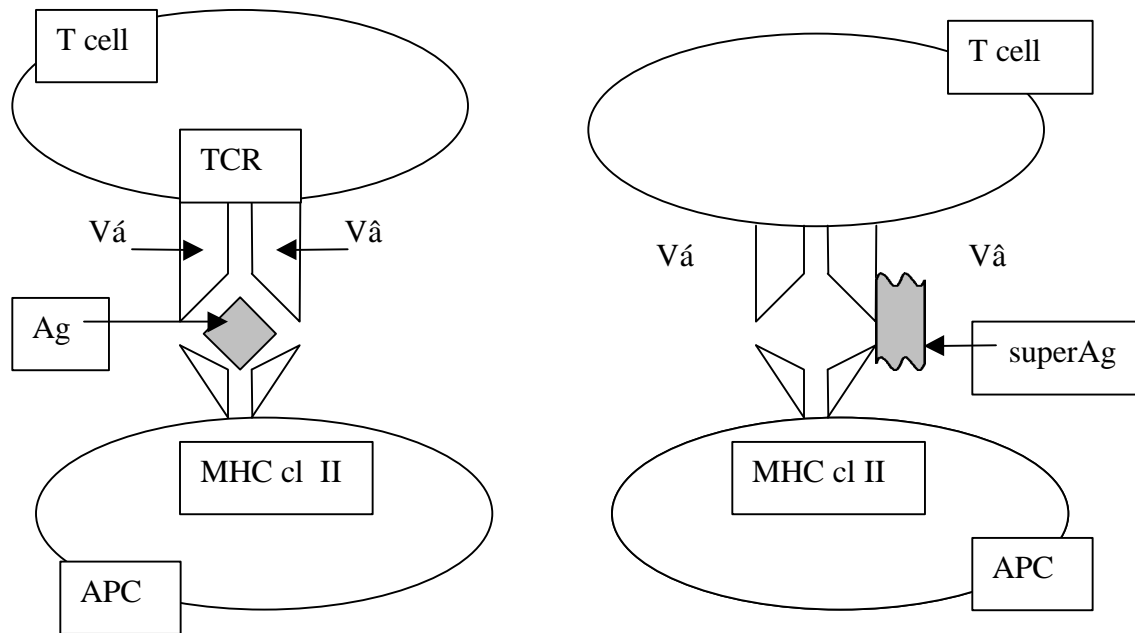


Figure 3:

T cell stimulation by superantigens

Recognition of antigenic peptide in MHC groove requires V α , J α , V β , D β , J β . On the contrary, recognition of superantigen requires only binding to V β and MHC class II.

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