Chapter A25

ENVIRONMENTAL INFLUENCES IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Abstract: Environmental factors, in particular infectious agents, are thought to have a major influence on the development and course of MS. Some of these influences are also reflected in the animal model, EAE. In this chapter, the role of infectious agents in the development and course of autoimmunity in EAE is discussed. Other environmental agents including trauma, solar radiation exposure, temperature, stress, toxins, are discussed in terms of their relevance to MS and EAE.

Key words: EAE, MS, infection, autoimmunity, trauma, solar radiation, UV light, temperature, stress, toxins

Introduction

Like several other diseases with an autoimmune mechanism and polygenic susceptibility, including type I diabetes mellitus and rheumatoid arthritis, the most commonly accepted working model of MS development is that it is an autoimmune disease triggered in genetically susceptible individuals by some environmental factor. MS thus arises as a result of an interplay of varying degrees of environmental and genetic factors. Such an interplay is important not only in determining the susceptibility of an individual to the disease but also possibly other aspects of the disease such as severity or course.

Experimental autoimmune encephalomyelitis (EAE) is in many respects the prototypical organ specific, T cell mediated autoimmune disease having provided many clues to the immune mechanisms of MS. As a useful animal model, EAE ideally must reflect some of these environmental influences that play a role in MS. Moreover, EAE being generally induced in strains of

animals, which are very well characterized immunogenetically, the genetic contribution remains equal across the strain or substrain, and the environmental conditions can be varied to provide clues to their contribution to the susceptibility and course of EAE.

Infectious agents

Of the many environmental factors implicated in MS, infectious agents have been most consistently investigated [1]. The possibility that MS is caused by an infectious agent has been entertained since the first detailed clinico-pathological description of MS, and is supported by several lines of evidence. The possibility that, in addition, infectious agents, directly or through their immunological consequences, may modify disease activity is also being increasingly substantiated by studies.

Many infectious agents have been implicated in MS, including spirochetes, bacteria, and viruses (table)

Table 1.

Some infectious agents linked to MS

Adenovirus
Borrellia burgdorferi
Canine distemper virus
Chlamydia pneumoniae
Coronavirus
EBV
Enterovirus
HSV 1, 2, 6, 8
Herpes zoster
HTLV-1 and 2
Measles
Mumps
Papovavirus
Rubella
Simian virus 5
Theiler's encephalomyelitis virus

Some of these infectious agents are linked to MS because of evidence of an increased titer of antibodies against them in the CSF of MS patients, for example measles and herpes zoster, or in their blood, for example EBV. Many, however, are linked to MS because of the existence of spontaneous or experimentally induced demyelination in animal models. These aspects are discussed in detail in the section of this book dedicated to viral models of

demyelination.

However, like in MS, and underscoring the utility of EAE as a model, infectious agents can influence aspects of EAE.

Self-reactive lymphocytes recognizing neuroantigens exist not only in MS patients but also in normal subjects. Moreover, the precursor frequency of these cells does not appear to be higher in MS patients. However, the neuroantigen-reactive T cells from MS patients are in higher state of activation than those of normal subjects [2]. Activated T cells are more capable of crossing the blood brain barrier and there, where they encounter their cognate antigen, they can cause the inflammatory demyelination damage. In relapsing MS, as in relapsing models of EAE, the reactivation of these cells may be a major event in the pathogenesis of relapses.

The role of infectious agents in these situations has been investigated, as it is recognized that infectious events (or immunizations) can trigger MS relapses or the first manifestations of the disease.

Several mechanisms may operate whereby autoreactive T cells can be activated and their action in EAE is discussed below.

Superantigen activation

Superantigens (SAG) are viral or bacterial products capable of activating a large number of T cells bearing the same T cell receptor (TCR)V chain [3]. SAG include several bacterial enterotoxins such as staphylococcal enterotoxin A (SEA) and B (SEB) and have been implicated in several human diseases. SAB bind the $V\beta$ region of the TCR outside the groove normally occupied by the antigenic peptide, and also the MHC class II molecule on the surface of antigen presenting cells. In EAE, and possibly in MS there may be a bias in the $V\beta$ TCR usage in particular in the early stages of the disease, before significant epitope spreading has occurred. SAG activation of neuroantigen-reactive T cells bearing the corresponding TCR may lead to a relapse in EAE. This is indeed the case. SAG SEB, which predominantly binds $V\beta$ TCR in mice has been demonstrated to induce relapses in myelin basic protein (MBP)-induced EAE in PL/J and PL/J x SJL/J F1 mice, whose MBP-reactive T cells predominantly use TCR $V\beta8$ [4, 5]. Moreover, we confirmed this ability of SEB to induce relapses [6]. In addition, we demonstrated that SEA (which does not bind $V\beta8$ in mice) also induces significant relapses. Therefore, although it was originally proposed that $V\beta8$ TCR confers specific tendency for autoimmunity [7], the ability of SAG to reactivate autoimmunity in EAE is not entirely dependent on $V\beta8$ TCR.

What is the mechanism by which SAG induce relapses? It is known that SAG preferentially activate Th1 cells [8, 9]. It is also known that superantigens induce a variety of proinflammatory cytokines and other

inflammatory mediators. We demonstrated that SAG induce IL-12, the major factor in the development of Th1-type immune responses. Indeed antibodies against IL-12 p40 blocked SAG-induced relapses in PL/J x SJL/J F1 mice. Moreover, IL-12 administration mimicked the effects of SAG, inducing itself relapses [6]. This result was also demonstrated in MBP-induced EAE in Lewis rats [10].

A similar response has been found with TNF. TNF administration induced relapses [11] while administration of neutralizing TNF antibodies partially suppressed relapses [4].

Several anti-inflammatory cytokines including TGF-β, IFN-τ, and IL-10 were capable of suppressing SAG-induced relapses of EAE.

It appears most likely that the mechanism of activation of potentially pathogenic T cells in EAE by superantigens, in addition to activation of a large number of T cells using the same TCR, involves tipping the balance of pro- and anti-inflammatory cytokines toward a more inflammatory environment.

Interestingly, SAG were not only shown to reactivate autoimmunity by inducing relapses in EAE, but also to modify susceptibility to EAE. Interleukin-6 (IL-6) is a proinflammatory cytokine also known to be induced by SAG. Besides IL-12 (p40) it is a proinflammatory cytokine whose deficiency is consistently known to render mice completely resistant to EAE [12, 13]. Administration of SEB to IL-6 knockout mice has overcome their resistance to EAE [14].

These results are highly relevant for MS. It is known that relapses or the initial attack are often preceded by infections. It is also known that SAG can activate human neuroantigen-reactive T cells [15]. This may represent an important mechanism whereby infectious agents can (re)activate MS. Knowledge of this possibility also has implications for the treatment of MS. For example, glatiramer acetate, currently successfully used in the treatment of relapsing remitting MS, promiscuously binds MHC class II inside the peptide binding groove but not requiring antigen processing [16]. Although initially developed as a mimic of MBP, it suppresses not only MBP-induced EAE but also PLP-induced EAE [17]. This promiscuous MHC binding makes it a potentially very useful immunomodulatory treatment, and it was shown to suppress autoimmune uveitis as well [18]. However, while it suppresses antigen-specific T cell proliferative responses in vitro to nominal antigen, it does not suppress SAG-induced responses, suggesting that its blockade of the peptide-binding site in the MHC class II groove, does not affect SAG binding. We have recently confirmed this observation in human SEB-stimulated T cells (Constantinescu C, Robins A et al, unpublished observations).

Molecular mimicry

Another possible activation mechanism for autoreactive T cells with potential to cause or reactivate autoimmunity is molecular mimicry. The concept implies the existence of a structural similarity and an immunological cross-reactivity between self antigens of the host and an infectious agents such as bacteria, viruses, or yeasts, which elicits an immune response, after which T or B cells developed against these agents respond against cross-reactive self-determinants. A most convincing and biologically relevant example of molecular mimicry in human autoimmunity and neuroimmunology is the Guillain-Barre syndrome (GBS) [19]. This is another autoimmune demyelinating disease of the nervous system involving the peripheral nerves. There is convincing evidence for a bacterial etiology in up to 50% of patients. Although typically monophasic, the presentation can be different (for example the various proportions of axonal, myelin, or cranial nerve involvement) and the distribution across age groups in different part of the world can also be different. *Campylobacter jejuni* is a common gastrointestinal pathogen that causes diarrhea. It also, less frequently causes GBS. However, up to two thirds of patients with GBS have serological evidence of a recent *C jejuni* infection. Of these serogroup Penner 19 is overrepresented, while, on the other hand, rarely associated with gastroenteritis. Different serogroups express different lipopolysaccharides (LPS) closely resembling human cell surface glycolipids. Infection with *C jejuni* can thus lead to production of anti-ganglioside antibodies, which cross-react with human glycolipids. The most common such cross-reactive self-antigen is ganglioside GM1. Anti-GM1 antibodies are found in approximately 70% of patients with C. jejuni-associated GBS and in about 30% of patients with GBS but without serological evidence of recent *C jejuni* infection.

Other anti-ganglioside antibodies are associated with specific clinical forms of GBS, for example anti-GQ1b with the Miller Fischer variant with prominent cranial nerve involvement and anti-GalNac-GD1a antibodies with distal motor variant. These antibodies can also be triggered by *C jejuni* as well as other infections.

Other infection can result in GBS, notably *Mycoplasma pneumoniae*. Molecular mimicry may play an important role here as well. It has been shown that *M pneumoniae* triggers anti-GalC antibodies, which, in high titre are associated with a demyelinating GBS. It is important to note here that these glycolipids are also present in abundance in the CNS myelin therefore may represent potential autoantigens in MS and EAE in addition to GBS. Moreover, EAE has been induced by immunization with GalC.

Although a significant component of neuropathology in GBS is antibody and complement mediated, a role for a T cell participation in molecular mimicry involving glycolipids, traditionally considered T-independent

antigens, is likely to exist, and this mechanism likely to be relevant to T cell mediated autoimmunity such as occurs in EAE and MS. It has been shown that GM1 antibody production by B cells of GBS patients is T cell dependent [20]. Moreover, self-glycolipids reactive T cells have bee found both in normal individuals and in patients with MS [21]. In addition, EAE is more severe when the immunogenic brain homogenate contains the glycolipid fraction than when this fraction has been removed [22]. Therefore, a T cell and B cell cross reactive immune response between infectious agent components such as LPS and myelin glycolipids is a conceivable mechanism whereby molecular mimicry generates and activates self-reactive lymphocytes in CNS inflammatory demyelination.

Another important example of molecular mimicry with great relevance to EAE and MS is the hepatitis B virus (HBV). Its polymerase has been found to have sequence homology and antigenic cross-reactivity with an encephalitogenic epitope on MBP. Moreover, immunization of animals with HBV leads to a histologically evident EAE-like inflammatory demyelination [23]. It is thus conceivable that T cells against antigenic determinants of the HBV polymerase recognize cross-reactive MBP epitopes and become self-reactive. Wucherpfennig et al screened viral sequences for potential homology with the peptide predicted to bind MHC class II groove of the commonest MS-associated haplotype, HLA-DR2 (DRB1*1501), and demonstrated that some of these peptides stimulated myelin antigen (MBP)-reactive T cells from patients with MS [24].

Bystander activation

Bystander activation is the mechanism in which T cells are activated by exposure to self-antigen in the situations in which an infection has created an inflammatory environment favorable for the development of Th1 type T cells, or in an evironment which allows the expression of an antigen normally not exposed to the immune system, for example a sequestered antigen. The latter situation is closely linked with the phenomenon of epitope spreading, which is known to occur in EAE and MS, whereby previously unexposed (cryptic) epitopes become exposed during the tissue damage accompanying the initial event [25]. The initial event may be the initial inflammatory demyelinating attack, but also potentially an event with another pathogenesis such as injury, ischemia, or indeed, infection. The latter can be exemplified by the fact that infection with viruses that cause experimental demyelination such as coronavirus or TMEV eventually leads to spreading of the epitope to self-determinants including the known neuroantigens of EAE such as MBP [26].

Another example of bystander activation with a sequestered antigen is that of αB-crystallin. This is a protein belonging to the heat shock protein family. It has been shown that αB-crystallin induces marked T cell responses

in individuals with MS, more prominenent than responses to other neuroantigens such as MBP or PLP [27]. αB-crystallin is normally sequestered, not exposed to the immune system, not being on the surface of oligodendrocytes, but the response against it may develop later in the course of the disease via epitope spreading [28]. Moreover, EBV has been shown to induce expression of B-crystallin in B cells, which then present it in an HLA-DR-restricted fashion [29]. Since serological evidence of EBV exposure is present in nearly 100% of people with MS [30], it is conceivable that a mechanism of activation of neuroantigen (in this case αB-crystallin)-reactive T cells is induction by this virus in the context of a non-sequestered environment.

The creation of an inflammatory environment for bystander activation of autoreactive T cells by infectious agents has been convincingly demonstrated (and it may in great part explained why in some EAE induction models there is need for adjuvants (see chapter on adjuvants in EAE). Mice transgenic for a TCR specific for MBP do not develop EAE when kept in a germ free environment; however, when placed in a germ-containing environment they develop spontaneous EAE [31]. It is known that bacterial products other than SAG are involved in this (re)activation of autoimmune disease. LPS of gram-negative bacteria is an example of such infectious product. It was shown to be involved in the reactivation of experimental arthritis by bacterial overgrowth in the bowel [32]. In EAE, CpG-rich bacterial DNA, which has been shown to have multiple immunostimulating activities largely centered on induction of proinflammatory cytokines, modifies resistance to disease of prototypically resistant mice, rendering them susceptible [33].

A common feature of these bacterial products is their ability to induce proinflammatory cytokines and in particular, IL-12, a key cytokine for the development of Th1 responses, required for EAE. Also adjuvants such as the *Mycobaterium tuberculosis*-based complete Freund's adjuvant (CFA) all induce IL-12 [34, 35].

A plausible working hypothesis, therefore, is that the final common pathway of activation of autoimmunity in EAE (and in MS) by infectious agents is through their effects on proinflammatory cytokines and in particular IL-12 and/or the related IL-23, with which IL-12 shares a subunit. In terms of how this applies to MS, it is likely that not all infections, but those infections preferentially inducing a Th-1-type immune response may trigger or reactivate autoimmunity. In support of this is the fact that the viral sequences found to have homology with HLA-DR2 binding motif capable of activating CNS-reactive T cells from MS patients belong to relatively common human viruses such as EBV [24]. A substantial proportion of people without MS have evidence of EBV exposure. Thus, a large number of HLA-DR2+ individuals would be expected to develop MS if that were the unique requirement for self-reactive T cell activation. The ability to build a

response in which these activated T cells have a Th1 phenotype, which may depend on a combination of host and pathogen factors may represent one of the additional requirements necessary for induction of autoimmunity.

An infectious agent capable of skewing the immune response toward Th2 may conceivably confer the opposite mechanism, protection against Th1 autoimmunity in EAE. It has been shown that coexistent Th2 responses dampen the mangnitude of a Th1 response. This may reflect the finding that the prevalence of asthma and allergic conditions is decreased in MS [36]. Some infections may also have a protective role against autoimmunity. This has been used to explain the increasing rates of autoimmune diseases including MS, paralleled by decreasing rates of infectious diseases in the developed countries [37].

Other possible mechanisms

Conceivably, other mechanisms may contribute to the pathology of EAE. Cytokines potentially induced by infectious agents may have directs effects on the CNS including oligodendrocyte apoptosis or permeabilization of the blood brain barrier.

Trauma

Whether head trauma can trigger the onset of MS or attacks of established MS remains controversial [38, 39]. However, it is known that head trauma can cause a breakdown of the blood brain barrier, thus potentially facilitating the entry of autoreactive T cells in the CNS. Recent experiments in EAE have shown that focal brain injury following the immunization can lead to more severe neuropathology, mediated in part by chemokines [40].

Solar radiation

A well-described feature of the epidemiology of MS is that its prevalence increases with increasing latitude. This holds true for both hemispheres [41]. Although in part this feature can be described on the basis of genetic factors taking into consideration various population migration patterns [42], it strongly suggests that an environmental factor linked to latitude plays a role in the susceptibility to MS. Of all potential factors investigated, the most consistently associated with MS has been the decreased level of exposure to solar radiation [43]. In addition, at the same latitude, there is an inverse altitude gradient, in that the prevalence of MS in mountainous areas is significantly lower than in neighboring regions in industrial valleys [44]. This further supports the concept that sunlight exposure has a protective effect against MS. Sunlight contains ultraviolet and visible light. Both have

the potential to affect MS and EAE.

Ultraviolet light

UV light from sunlight has numerous biological activities including many effects on the immune system. It is therefore used as a therapeutic agent in a number of diseases. It induces apoptosis in several cell types [45]. It also modifies properties of lymphocytes potentially changing the autoreactive cells and rendering them tolerant. Photopheresis, a procedure in which circulating blood cells are exposed extracorporeally to UV light after administration of a photosensitizing agent, and then reintroduced in the circulation, is based on this principle. Although established in cutaneous T cell lymphoma and graft versus host disease, photopheresis has had conflicting results in MS. In an uncontrolled report, two patients with relapsing remitting disease benefited [46], while in a placebo-controlled study in progressive MS it was not beneficial [47]. In EAE, a recent study shows that photopheresis decreases the number of relapses in a relapsing model in rats.

A major role of UV light is in the supply of the Vitamin D. Diet, in particular fish and vitamin D-enriched milk provide only a part of a human's daily requirement of Vitamin D. The majority of this requirement is provided by the Vitamin D synthesis in the skin. Pre-vitamin D3 is synthesized in the skin from 7-dehydrocholesterol in a reaction catalyzed by UV light. It then isomerizes to form Vitamin D3, which is then converted to 25-hydroxy-Vitamin D3in the liver and then to the active 1,25 Dihydroxy-Vitamin D3 (calcitriol) in the kidney.

Goldberg, noting a high prevalence of MS in areas with low solar radiation exposure suggested that this might be related to low vitamin D3, and that this vitamin is important in myelin biosynthesis [43]. There is no evidence for a role of vitamin D3 in myelination, but there is increasing evidence for its multiple roles in regulation of immune functions. It has been shown that vitamin D3 and its biologically active derivatives suppress the major Th1 inducing cytokine, IL-12 [48]. Thus, its deficiency may lead to an enhanced Th1 immune response. The epidemiological evidence suggesting that MS patients may have a vitamin D3 deficiency was confirmed in studies in which 25-hydroxy-vitamin D3 was actually measured in MS patients. These showed a very significant proportion of vitamin D3 deficiency or insufficiency. Moreover, there was evidence of significantly reduced bone mass in women with MS compared to age-matched controls [49].

In EAE, Cantorna et al [50] showed that oral administration of calcitriol

prevented the onset of the disease, and its administration at the first clinical signs of EAE suppressed further progression. In further studies [51] the same group showed that calcitriol also is capable of reversing the clinical signs of disease in mice that already had clinically severe disease.

The mechanisms of action of calcitriol in EAE are not entirely elucidated. However, it is likely that its acts on the balance between pro- and anti-inflammatory (Th1/Th2) cytokines in favor of the latter. Cantorna et al [51] presented evidence that calcitriol induces anti-inflammatory cytokines TGF-β1 and IL-4. On the other hand, another study demonstrating suppression of EAE by vitamin B12 showed that the major mechanism was inhibition of IL-12 [52].

Vitamin D3 is not only effective in EAE. It was also successfully used in other experimental models of autoimmune disease including collagen-induced arthritis (where it was effective in both preventing and suppressing disease), [53] and insulin dependent diabetes mellitus in NOD mice [54]. It also has a beneficial effect in ameliorating the human inflammatory, presumed autoimmune disease, psoriasis [55].

Visible light

A fundamental component of sunlight is, of course, visible light. There is evidence that visible light is also important in MS and EAE. Daylight is also closely linked with the biological clock. One possibility explaining the latitude gradient for MS is that day length may be related to MS prevalence. There is anecdotal evidence of relapses being more frequent in the spring and autumn. The biological clock that maintains daily and seasonal rhythmicity may have an influence on the susceptibility to and manifestations of, autoimmune disease including EAE and MS.

In mammals the central clock is in the suprachiasmatic nucleus (SCN), which sends signals to the pineal gland to produce its hormone, melatonin, in a cyclical fashion. Melatonin feeds back onto the SCN and can modulate the clock cycle [56]. Melatonin can also modulate the body temperature cycle. The body temperature is high during the day, when melatonin levels are low [57].

Melatonin has a number of immune effects, in particular immunostimulatory activities [58]. It enhances production of IFN-γ and appears to favor a Th1 bias of T cells. Interestingly, this action has been shown to be due to the ability of melatonin to induce the major cytokine driving Th1 responses, IL-12 [59]. In a study of diurnal rhythmicity of cytokine production in humans, the analysis of the ratio of IFN-γ to IL-10

indicates that the strongest Th1 bias coincided to the highest levels of melatonin [60]. Removing production of melatonin by pinealectomy can suppress experimental autoimmune disease including collagen-induced arthritis. This has subsequently been demonstrated in EAE as well [61].

The peak production of melatonin is at night and the nadir is during daytime. Visible light suppresses production of melatonin. Thus conceivably increased daylight exposure time may be associated with lower susceptibility to Th1 mediated autoimmunity, in part possible due to decreased melatonin-enhanced Th1 cytokine production. This may explain why the geographic distribution of MS is related to the degree of sunlight exposure.

In experimental models of autoimmune diseases, this has been confirmed: constant light ameliorated experimental arthritis. In addition, exposure of animals with EAE to constant visible light significantly suppressed disease activity [62].

Melatonin can act directly upon cells of the immune system including T and B cells which have been shown to possess melatonin receptors [57]. We have used a melatonin receptor antagonist, Luzindole, injected at nighttime in the dark during peak melatonin production, and significantly suppressed EAE disease activity [63].

These results concur in suggesting that sunlight' visible light spectrum via suppression of melatonin, and UV light spectrum via provision of vitamin D3, and their effects on the immune system may contribute to the susceptibility to and manifestations of EAE and MS.

An additional and opposing aspect of melatonin needs to be considered. By modulation the body temperature clock, it lowers the body temperature. This has led to relief of several temperature-dependent symptoms in demyelinating disease. It is known that increases in body temperature can worsen symptoms of MS, usually in a transient fashion (Uhthoff's phenomenon). Conversely, lowering the body temperature improves symptoms of MS, possibly by improving conduction through demyelinated nerve fibers. This has been shown in human and experimental demyelination. Thus melatonin, which lowers the body temperature, can lead to short term symptomatic relief of MS symptoms. In the long term, however, its immunostimulating, Th1 biasing effects may make this hormone detrimental, and the above evidence of the positive effects of its suppression on EAE supports this.

Although speculative, another aspect of sunlight exposure may be protective against inflammatory demyelination: its ability to stimulate

melanin production. Even within the same latitude, dark skinned individuals are at lower risk of developing MS than light skinned individuals. In EAE, with notable exceptions, susceptible strains are albino, while resistant ones are dark. This may be related to the protective effect of melanin, for example its known ability to scavenge and suppress free radicals.

Stress

Stress can be physical (trauma) or psychological (emotional). This part of the discussion refers to the latter. The role of stress in MS remains controversial. It is very difficult to assess and quantify stress. Many differences in the threshold, degree of perception, and cultural responses to stress between individuals are likely. Stress is so much a part of everyday life that envisioning a stress free environment is difficult in humans. Because of the ubiquity of stress, prospective studies in MS linking disease activity to stressful events are also difficult, and retrospective studies pose problems with controls. As with major physical trauma, major stressful events are most easily remembered, which may bias retrospective studies. However, a study in MS has suggested that stress may have a protective effect. Investigating the frequency of relapses and new onset of MS in Israel during the period of general stress to the whole population of Israel, of the Gulf War, Nisipeanu and Korczyn [64] have shown it to be significantly lower than expected. On the other hand, stress is frequently blamed, usually by patients themselves, for the onset of MS or for an exacerbation in the MS symptoms.

In solving this apparent discrepancy, here again animal models can be very helpful. Although emotional stress in humans is obviously a very complex phenomenon, its simplified direct biological correlate is a strong sympathetic response. The immunological phenomena that ensue are mediated by the presence of both beta2 and alpha2 adrenergic receptors on cells of the immune system. It is thought that Th1 cells possess beta2 receptors and thus are more amenable to immunomodulation via these receptors, while Th2 cells do not. Therefore, creating conditions representing the biological correlate of a stress free environment in an experimental animal can be accomplished by sympathectomy. Chemical sympathectomy can be achieved by using 6-hydroxydopamine hydrobromide, which becomes oxidized in the sympathetic nerve terminals and destroys them resulting in long term sympathectomy in the neonate and temporary axotomy in the adult. The short-term results of either procedure are similar, and consistent with a significant enhancement of immune functions.

This can be reflected in EAE as well. Animals having undergone

permanent or temporary sympathectomy have been shown to exhibit more severe CNS inflammation [65, 66]. In adoptive transfer experiments between sympathectomized and non-sympathectomized mice, it has been shown that donor T cells from sympathectomized animals induce more severe EAE, and when the recipient is sympathectomized, the EAE is also more severe, suggesting that both T cells and APC from sympathectomized animals contribute to the increased severity of EAE [67].

In addition to sympathectomy, experimental situations inducing stress in the animal have been tested for its effect on EAE. These have included maternal deprivation, a swimming test, thermal stress etc. These investigations have consistently shown a protective effect of stress on EAE.

Taken together, these results suggest not only that EAE reflects reliably the relationship between MS and stress, but also that sympathectomy as is reliable as a surrogate for creation of "stress-free" conditions while adrenergic stimulation mimics stress.
This is further confirmed by the fact that beta adrenergic agonists such as terbutaline or isoproterenol mimic stress by inducing similar, suppressive changes in the immune system. These effects were also seen on EAE [68].

After the initial sympathetic stimulation and immune suppression by stress, there appears to be an overshoot of immune stimulation as stress declines or the stressful situation has passed. It has been shown that after exposure of medical students to examination stress with the corresponding immune suppressive effect, there was a hyper-responsiveness in immune functions 4-6 weeks after the examination. It is also possible that chronic stress induces different changes than acute or one-time stressful conditions. This may reconcile the discrepancies between the studies showing a protective effect of stress on EAE and MS and situations in which worsening or onset of MS were attributed to stress.

Diet

Besides the importance of vitamin D3 (with the contribution of diet and sunlight) as discussed above, there are several other associations of diet and MS, which, despite much investigation, remain controversial. The most consistent aspects linking diet and MS are the positive association with the consumption of meats (in particular those preserved by curing or smoking) and the prevalence of MS, and the inverse relationship with the use of fish and vegetable oils rich in polyunsaturated fatty acids. The protective effect of omega-6 and omega-3 fatty acids has been investigated in double blind trials. They appear to decrease the severity of relapses. They reduce production of proinflammatory cytokines and eicosanoids [69]. Similarly,

omega-6 fatty acids have a beneficial effect in EAE. This effect appears to be mediated by enhancing TGF-beta1 and prostaglandin (PG) E2 production [70].

In addition to vitamin D3, retinoids such as vitamin A appear to be important in skewing the immune response toward a Th2, anti-inflammatory type. In experimental animals, a diet poor in vitamin A resulted in an enhanced Th1-type responses and administration of retinoids led to suppression of EAE. The mechanism has been shown to be via suppression of IL-12 and enhancement of IL-4 production. [71-73].

Toxins

Several toxins have been implicated in MS but none have been consistently and definitively demonstrated to have role in MS. The more notable ones have been occupational exposure to Zinc and organic solvents.

With regard to EAE, these have not been studied extensively. However, several toxins have been shown to induce experimental demyelination, and, although not necessarily involved in human demyelinating disease, provide important insights into the pathology or demyelination, the biology of myelin producing cells and the glial and neural response to demyelination. They can also answer important questions regarding mechanisms of remyelination and the use of novel mechanisms to induce or enhance it.

Several toxin-induced demyelinating models exist.

Cuprizone-induced demyelination

The drug Cuprizone (biscyclohexanone, oxaldihydrazone) represents an excellent model fro demyelination [74-78]. When the drug is added to the diet of weanling mice for several weeks or longer, a near-total, primary demyelination of the superior cerebellar peduncle develops. The demyelination is caused by oligodendrocyte degeneration, but there is relative preservation of axons. There is a continuum between myelin degeneration via formation of vacuoles and death of oligodendrocytes. The prevalence of these pathological processes is in part dependent of the concentration of the toxin, suggesting that the demyelination via vacuolation does not represent a specific myelinopathy but rather an dose-dependent oligodendrocyte toxicity. The myelin debris is removed by microglia/macrophages and astrocytes. The relatively preserved axons become invested in glial processes. There is a remarkable glial response to demyelination, characterized by the occurrence of immature glial cells, both of astrocytic and oligodendroglial nature. There is also survival of some of the mature oligodendrocytes.

A great advantage of this model is that it allows the detailed study of the kinetics and mechanisms of remyelination in the CNS.

With the re-institution of a normal, cuprizone-free diet, remyelination begins within a week, and continues until nearly all residual axons are remyelinated. The pattern resembles that of myelination as part of the normal CNS development, with spiral wrapping around axons. Cellular sources of remyelination largely represent residual surviving adult oligodendrocytes and new cells differentiated from immature precursor forms. The possibility of perineuronal satellite cells contributing to remyelination has also been proposed. Like in EAE and MS, remyelination is incomplete: the maximal thickness reached by new myelin sheets is ½ that of normal sheets, and the internodal distance is shortened.

Importantly, recurrent demyelination can be demonstrated in this system. This can be accomplished by repeated exposure to cuprizone. This recurrent demyelination appears to be more protracted and the remyelination potential is more reduced. This is attributed to reduced numbers of surviving oligodendrocytes. The intensity of the glial reaction to the injury is lower compared to the initial reaction, and this may also in part explain the less efficient remyelination. In addition, this model of re-exposure to cuprizone shows that remyelinated axons are not more susceptible to demyelination than axons that have not previously been demyelinated. This finding, if it can be extrapolated to other mechanisms of demyelination, has important implications for EAE and MS.

Ethidium bromide-induced demyelination.

Another model of toxin-induced demyelination is the ethidium bromide exposure model [79]. This is accomplished by the intracisternal injection of ethidium bromide in rats. The toxin induces status spongiosus and oligodendrocyte degeneration. Many axons are demyelinated within 6 days of injection. The myelin debris is phagocytosed largely by microglia/macrophages. Such cells also infiltrate between myelin lamellae. There can be complete oligodendrocyte degeneration in areas of myelin loss.

There is remyelination in this model as well. Remyelinated axons are thinner. In addition, Schwann cells may contribute to remyelination, a feature recognised in some forms of MS with predominanlty myelopathic features in Japan.

There are additional models of toxin-induced demyelination including diphtheria toxin and lysolecithin induced demyelination [80]

Effects of temperature

The effects of temperature on clinical and experimental demyelination have been investigated since the report by Uhthoff of worsening symptoms

of optic neuritis associated with an increased in body temperature caused by physical exertion [81]. It is now recognized that any other mechanisms of elevating the body temperature (even within the normal range, even by as little as 0.5C) can cause an increased perception of symptoms of demyelination (Uhthoff phenomenon), and that such symptoms are not limited to visual disturbances [82].

Conversely, a decrease in body temperature can improve symptoms of demyelination. This has been shown in humans with optic neuritis given ice cream or iced water [83, 84]. Although Uhthoff's phenomenon is not exclusively limited to primary demyelinating conditions, having been reported in giant cell arteritis or brain tumors, the ice test seems to allow distinguishing optic neuritis from other clinically similar conditions, and has been suggested as an auxiliary differential diagnostic tests.

These phenomena have been studies in experimental models of demyelination as well.

Lower temperatures increased conduction while high temperatures impair it . [85-87]

Hyperbaric oxygen

Although hyperbaric oxygen has long been advocated by some authors as a treatment for multiple sclerosis, no convincing evidence from controlled trials has been obtain to support its use. There are anecdotal accounts of symptomatic improvement with hyparbaric oxygen treatment.

Reports in EAE are also somewhat controversial. Theoretically the high pressure provided during hyperbaric treatments can affect the blood brain barrier and increase its permeability, thus potentially facilitating invasion of CNS by myelin-reactive cells. However, an opposite effect on the blood brain barrier has been proposed.

Dysbarism, as occurs in scuba diving accidents, may result in permeabilization of the blood brain barrier and autopsies of fatal cases have shown demyelination in the spinal cord similar to that seen in MS and EAE.

Few studies have investigated the effects of hyperbaric oxygen treatment in EAE. The effects may be dual, and may depend on the timing of the treatment relative to the immunization.

An old study revealed immunosuppressive effects of hyperbaric oxygen, with an associated positive effect on EAE in guinea pigs [88]. These effects depended on the duration of treatment and gas pressure. They, were, however, sustained only briefly after discontinuation of treatment. The immunosuppression was demonstrated by inhibition of cellular reactivity to myelin basic protein and tuberculin.

More recent studies using magnetic resonance (MRI) imaging showed that hyperbaric oxygen treatment does not reduce the disruption of the blood

brain barrier or the cerebral edema seen in EAE [89]. On the contrary, it was shown to increase the blood brain barrier breakdown. When given at the time of immunization as a preventive treatment, it caused amelioration in the clinical course of EAE, but when given 11 days after immunization in attempt to modify EAE, it had no effect [89].

Conclusion

In conclusion, the various environmental influences on MS can be investigated in the animal model, EAE. The mechanisms by which infectious agents can have an impact on MS and autoimmunity in general can be carefully dissected in the EAE model. Other factors such as UV and visible light exposure, stress, temperature, trauma, and their relevance to MS can be explored.

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