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# T Cell Approach to Demyelinating Diseases

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## Introduction

In an early morphologic description of the multiple sclerosis (MS) lesion, Charcot drew attention to two cellular changes within demyelinated brain plaques: round cell infiltrates around post-capillary small venules and astrocytic activation [16]. Both changes were found particularly in areas with apparent active cellular interactions and much less often in the centers of the lesions which were composed mainly of "sclerotic" glial scarring tissues.

From the earliest days of MS research till today, the primary aim was to logically interconnect demyelination, cellular infiltration, and astrocyte activation in order to retrace a comprehensive pathway of this complex disease. Due to remarkable technical progress during the past few years in the characterization of lymphoid cells, of their culture in vitro, and in combining immunologic techniques with novel approaches in neurobiology, it has become possible to experimentally approach this problem. In this article, we will summarize recent data which are the basis of a concept of T cell contribution to autoaggressive diseases in the nervous system. We will tentatively delineate pathogenetic stages, and finally discuss possible therapeutic approaches arising from the novel results.

## 1. Autoimmune Demyelination?

A number of findings have been cited in favor of autoimmune mechanisms acting in demyelination of MS. They include disturbances of T cell subset equilibria in the peripheral blood (this will be dealt with by Bach in this series), reduction of the activity of NK cells, and demonstration of myelin-basic protein (MBP) reactive T cells in the peripheral blood of MS patients [5, 15]. Perhaps the most convincing

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argument, however, is the peculiar histologic pattern of the inflammatory reaction in the MS plaque. It has been shown recently that at least in the early, active stage of plaque formation, most of the infiltrating cells are T lymphocytes [31]. These infiltrates are almost indistinguishable in pattern and cell distribution from experimentally induced lesions of autoimmunized animals. Various models of experimentally induced autoimmune inflammation of the nervous system have been extremely informative for better understanding certain stages of MS. None of these models represents the whole disease; each one reflects only one more or less restricted aspect of the pathogenesis.

Inflammatory autoimmune demyelination has been induced in a number of experimental systems. Most of them were based on injection of susceptible animals with spinal cord homogenate emulsified in complete Freund's adjuvant (CFA). In several models, actual demyelination coincided with a chronic and relapsing course of the clinical disease. This is true for strain 13 guinea pigs, SJL mice, and for certain rats [6, 17, 24].

The actual antigenic conditions leading to demyelinating experimental autoimmune encephalomyelitis (EAE) are unknown. It appears that a combination of different myelin antigens is required. An encephalitogen, such as MBP seems to be necessary to cause autoimmune inflammation, and thus to permeabilize the blood-brain barrier (vide infra). In addition, distinct autoantigens, such as galactocerebroside, may elicit immune responses which then lead to actual demyelination [23]. Humoral autoantibodies may be involved in this later step [12].

Interestingly, virus encephalitis models have been described recently, which in their chronic phase closely resemble chronic relapsing EAE and which appear to involve autoimmune components subsequent to a primary virus attack. One of these models is Theiler virus infection of mouse brains, which features an acute viral attack of the central nervous system (CNS), which involves demyelination [14]. In a later, chronic phase, demyelination is associated with apparent autoimmune infiltration around perivascular areas. At present the actual target autoantigen in Theiler EAE us unknown. Lymphocytes from infected mice were found to respond to myelin preparations, but not to MBP (P. Lampert, personal communication).

A similar model involves Lewis rats infected with JHM strain corona viruses. As in Theiler disease, the rats undergo an acute viral encephalitis, which is followed by a chronic EAE-like disease. Demyelinating plaques are seen around postcapillary venules. In the JHM Lewis rat model, lymphocytes were isolated, which in vitro responded to guinea pig MBP, and which upon reactivation in vitro, were able to transfer acute, non-demyelinating EAE to naive syngeneic recipient rats [33].

A feature common to all models of demyelinating EAE are perivascular infiltrates in the centers of the lesions. Recent immunocytochemical analyses demonstrated that in EAE infiltrates T lymphocytes predominate, and among them, T cells with the membrane phenotype of helper cells or effector cells in delayed type hypersensitivity (DTH) reactions [29, 30]. Also in most cases where EAE was transferred, T lymphocytes of the T-helper/DTH effector phenotype were most probably the active cell [20]. Recently, a demyelinating disease could be transferred by T cells [18]. This strongly suggests that at least one critical prerequisite for autoimmune demyelination is autoaggressive T helper/DTH cells. It appears,

furthermore, reasonable to extrapolate from these data to the pathogenesis of human demyelinating disease.

Demyelination concomitant with autoimmune reactions can be brought about by different mechanisms. Specific immune factors can be responsible for the destruction of the myelin sheath and myelinating cells. Thus, it has long been known that antibodies against myelin determinants can destroy myelin in vivo and in vitro. Autoantibodies against galactocerebroside and whole myelin, but not anti-MBP antibodies were predominant demyelinating immunoglobulins [26]. Much less clear is the contribution of myelin-specific cytotoxic T lymphocytes, which potentially could demyelinate by directly attacking and lysing myelinated membranes. Experimental support for myelin-specific cytotoxic T lymphocytes comes from the immunocytochemical demonstration of T lymphocytes with "suppressor/cytotoxic T cell phenotype" in EAE or MS lesions. Except for one study, where in actively induced EAE of strain 13 guinea pigs, MBP-specific cytolytic T cells were demonstrated lysing MBP-coated syngeneic macrophages [1], little convincing evidence for the existence or role of myelin-specific cytolytic T cells has been provided so far.

On the other hand, T lymphocytes could nonspecifically lyse myelinated cells in the vicinity of antigen-presenting cells. This "innocent bystander effect" which could be due to soluble lymphotoxins was thought to be responsible for demyelination in tissue culture [35]. It cannot be ruled out that this phenomenon has also some role in vivo, but it should be emphasized that bystander demyelination would be confined to the immediate surrounding of the actual immune reaction, and therefore cannot easily account for formation of a whole demyelinating plaque. In addition, myelin can be destroyed by mechanisms not directly related to immune effector mechanisms. Thus, in autoimmune neuritis, it has been shown that the edema caused by the autoimmune infiltrations can raise the intraneural pressure to a point to cause local ischemia [22]. Ischemic demyelination would be a secondary effect of the EAN response. It is open whether under the less rigid anatomic conditions of the brain, edema-dependent pressure increases could have a substantial role in demyelination.

Finally, it has been recognized recently that myelin itself without specific antibody binding can activate the classical complement activation pathway [7, 32]. To date it is open, whether this mechanism can lead to demyelination in situ. If so, demyelination secondary to leakage of the blood-brain barrier, implying penetration of serum complement to the myelinated surfaces, could be an important ancillary mechanism in CNS lesion formation.

Not all functional defects in MS can be explained by demyelination solely. It is typical for MS that some neurologic deficits occur so fast and subside so rapidly that demyelination and remyelination cannot account for them. Similar to acute EAE, where neurologic symptoms occur in the absence of demyelination, nerve conduction may be impaired be either physical effects of edema formation, or by factors formed within the inflammatory infiltrates. A so far unrecognized mechanism of inflammatory disturbance of CNS nerve conduction was recently noted by Yarom et al. [36]. They found that myelin-specific T line cells can interfere with conduction in optic nerve explants within a few minutes. This block was reversible: after removal of the T lymphocytes, optic nerve conduction was resumed. Furthermore,

the interaction was immune specific. Only myelin-specific T line cells blocked optic nerve function, and they acted only upon syngeneic, Ia-compatible tissue. The mechanism of this novel effect of immune cells on nervous function, and its relevance for clinical disease are incompletely understood at present. It is, however, possible that similar effects actively contribute to the generation of symptoms in multiple sclerosis and autoimmune encephalitis.

## 2. Isolation of Autoaggressive T Lines

The most direct approach to study the role of T cells in encephalitic diseases is to select T cell clones from encephalitic animals and to establish permanently growing T lines with determined antigen specificity. This approach resembles generation of monoclonal antibodies in two aspects. First, as monoclonal antibodies, permanent T lines are absolutely pure immunologic reagents. Second, due to their permanent growth, theoretically unlimited amounts of the cells can be prepared. A unique advantage is, however, that the lines are not phenotypically transformed, and will be fully reintegrated into the host's immune system when transferred.

MBP-specific T cells were first isolated from Lewis rats in 1978 [34]. In the following years, Ben-Nun et al. succeeded in developing a tissue culture system which permits establishment of permanently growing, clonable T cell lines from Lewis rats at an unprecedented high yield [3]. The essentials of this technique are based on two principles: activation of the antigen-specific T cell clones with antigen by presenter cells and their propagation using growth factors. Several selective pressures are applied. First, rats are immunized with MBP/CFA which leads to an in vivo increase of T cells specific for the antigen MBP, but also for the PPD component of the CFA. Second, lymph node cells of the primed animals are restimulated in vitro with MBP which leads to blast transformation, expression of receptors for growth factors (interleukin 2, Il-2), and proliferation of the specific T cells. Third, the activated specific T blasts are separated from the irrelevant non-stimulated T cells on discontinuous density gradients. Finally, isolated T blasts are cultured in the presence of IL-2 which keeps them in rapid proliferation. Due to gradual loss of the Il-2 receptor during this expansion period, the proliferation rate eventually slows down and the T blasts revert back to small resting cells. In this stage the cells can be restimulated with antigen on presenter cells. This cyclic interchange of restimulation and expansion not only yields rapid selection for and expansion of T cells specific for the priming antigen, but also is the basis for monitoring the specificity of the T cells. Table 1 illustrates, how after only a few restimulation cycles, the T cells become exclusively specific for MBP and completely loose reactivity to the PPD component in the priming step. T lines provide a powerful tool to in vitro manipulate T cell activation by varying antigen presenting cells, antigen subfractions, blocking antibodies, and genetic combinations. Even more important, the activated T blasts are highly encephalitogenic when injected into normal syngeneic rats. Thus, the effector part of the T cell mediated autoimmune reaction can be studied separately in a highly specific and experimentally accessible way.

**Table 1**[a]. Specific and syngeneic presenter requirement in the in vitro EAE memory response

| Responders[b] | Presenter[c] | Stimulant[d] | | |
|---|---|---|---|---|
| | | BP | PPD | ConA |
| Primed Lewis | – | 15826 | 18251 | ND |
| Naive Lewis | – | 1224 | 7397 | 103569 |
| 2° anti BP | – | 4735 | 812 | 16564 |
| 2° anti BP | BN irradiated | 4820 | −835 | 31650 |
| 2° anti BP | Lewis irradiated | 26924 | 1469 | 22789 |

[a] From the original publication [20] in 1978, with permission of Elsevier North Holland Inc
[b] Fresh primed or naive Lewis lymph node cells ($1 \times 10^6$ cells/well) cultured for 84 h, or Lewis 2° anti-BP cells ($1 \times 10^5$/ml) cultured for 60 h
[c] Naive lymph node cells, irradiated (2000 R)
[d] BP: Basic protein crude extract (10 μg/well); PPD (10 μg/well); ConA (5 μg/well), $^4$H-thymidine incorporation (cpm): net values (experimental groups — unstimulated background); ND: not done

## 3. Ia Antigens and Autoimmune T Lymphocytes

A major breakthrough in cellular immunology was reached in the early seventies when the studies of Zinkernagel and Doherty [37], and of Shevach and Rosenthal [27] established that T lymphocytes, in contrast to B lymphocytes, are unable to recognize and to react against an antigen alone. It became clear that in order to elicit an immune response, any antigen must be presented on a cell surface, and there it must be recognized in the context of determinants of the major histocompatibility gene complex (MHC). T helper/DTH lymphocytes recognize antigen in the context of Ia (class II) MHC determinants, whereas cytotoxic T cells recognize antigen only along with transplantation antigen of class I. MHC restriction is imposed on conventional foreign antigens, as well as on autoantigens. In the case of encephalitogenic rat T line cells, it has been found that all cells are restricted by Ia antigens. This is not surprising as all T lines expressed the rat T helper/DTH marker W3/25, and not the marker for cytotoxic cells OX8.

The role of Ia antigen in the pathogenesis of autoimmune disease is highly complex. First and foremost, by their function as a restriction element in antigen presentation, Ia determinants are centrally involved in genetic control of autoimmune susceptibility. However, as will become clear later, Ia determinants seem to have further roles in intercellular interactions, which are involved in the pathogenesis of autoimmune diseases. Consequently, as will be discussed later, manipulation of Ia determinants most probably will become a novel and promising approach to therapeutically influence autoimmune disease. As already mentioned, encephalitogenic MBP-specific T lymphocytes recognize their antigen, MBP, only in the context of self Ia determinants. This was demonstrated using antigen-presenter cells of different MHC-recombinant and -congenic rat strains (Table 2).

Within a few hours after recognition of antigen, the T line cells transform to activated large lymphoblasts and enter into rapid proliferation. Immediately with morphologic activation the T line cells, which in the quiescent state do not express demonstrable Ia antigens on their surface, acquire significant amounts of membrane

**Table 2.** Genetic restriction of MBP presentation to the MBP-specific T cell line BS.BP[a]

| Presenter cells[b] of strain | RT 1[d] ABC | 3H-thymidine incorporation (cpm) | |
|---|---|---|---|
| | | No antigen | MBP[c] |
| BH | 1 1 Lv3 | 201 | 12732 |
| LEW.1Lv3 | 1 1 Lv3 | 513 | 15885 |
| BH.1L | 1 1 1 | 317 | 30232 |
| LEW.1LM1 | 1 1– | 352 | 16172 |
| LEW.R15 | 1 1 u | 411 | 18144 |
| LEW.R14 | u u 1 | 174 | 227 |

[a] The BS.BP T cell line was raised from BS rats (RT1 compatible with BH); $10^4$ cells/well
[b] Thymus cells, 2000 rad irradiated; $10^6$ cells/well
[c] Myelin basic protein; 10 μg/ml
[d] RT1: major histocompatibility complex of the rat. *A* locus coding for class I antigens *B* locus coding for class II antigens *C* locus coding for class I antigens (outside the RT1 complex proper)

bound Ia. In fact, expression of Ia on T lymphocytes has been regarded as an activation marker in several species, including man and mice. In an attempt to determine the cellular origin of the newly expressed T blast Ia, T line cells were activated either by MBP presented on syngeneic presenter cells or by the polyclonal mitogen Concanavalin A (ConA) in the presence of syngeneic or allogeneic presenter cells. A cytofluorometric analysis using a suitable panel of monoclonal antibodies against monomorphic and polymorphic Ia determinants demonstrated that in each case, the Ia phenotype of a T blast corresponded to the genotype of the presenter cells, and not to the genotype of the T line. Therefore, the Ia antigens transiently expressed on activated T line cells must be derived from the presenter cells, rather than being the biosynthetic product of the T line cells themselves.

The physiologic role of the Ia antigens on the T blast is obscure. Acquired Ia certainly does not determine specificity in the efferent phase of EAE generation. T blasts were "disguised" with allogeneic Ia antigens in ConA cultures containing allogeneic presenter cells. The blast were then transferred in rats which were genetically identical with either the T lines, or with their adopted Ia. The lines, without exception, transferred clinical EAE only to T line-compatible rats (unpublished observation).

## 4. T Lymphocytes and the Blood-Tissue Barriers

Autoimmune inflammatory diseases of the nervous system are the result of a pathologic interaction between two organ systems which both act throughout the entire organism. It is conceivable that the initial site of autoimmunization in human as in experimental disease is not identical with the site of actual autoaggressive tissue destruction. It is even possible that autoimmunization is triggered outside the nervous system. This is certainly the case in experimentally induced autoimmune encephalitis (EAE) and neuritis (EAN) models, where autoantigen is inoculated

subcutaneously into foot pads and where the activated autoaggressive T lymphocytes must find their way to the actual target tissue, i. e., CNS or nerves. Target homing is especially impressive in EAE and EAN transferred by autoimmune T line cells [13]. These T line cells are selected for recognizing one myelin component, and depending on the tissue origin of the myelin determinant, the line cells will selectively cause either peripheral EAN or central EAE. Target specificity is a programmed property of the cells, and is independent of possibly co-transferred exogenous autoantigen, since the specific target pattern will be formed irrespectively of whether the line cells were activated by the nominal autoantigen presented by syngeneic cells, or by a T cell mitogen presented by allogeneic or syngeneic presenter cells.

To appreciate the target selectivity of the T line cells, it is helpful to note that the PNS, and even more so, the CNS is separated from the blood circulation by a tight barrier, the blood-brain barrier (BBB) or blood-nerve barrier (BNB). In the central nervous system, the BBB consists of three layers. The first layer is formed by endothelial cells interconnected by completely sealing tight junctions. This inner ring is surrounded by a subcellular basal membrane, which in turn is surrounded by a tightly felted meshwork of astrocyte processes.

The BBB, due to its particular anatomic structure, is known to be a reliable barrier preventing blood-borne substances and cells from freely entering the CNS parenchyme. Recent experiments done in collaboration with H. Lassmann and K. Kitz have suggested that interaction of the BBB with activated T cells is much more active and complex. Rather than being a mere physical barrier for T blast cells, the BBB seems even to be required for T lymphocytes to enter the CNS. Activated MBP-specific T line cells, which are given intravenously and which unfailingly will induce EAE in host animals, do not transfer EAE at all when given intrathecally, i. e., by-passing the BBB. Thus, the BBB seems to have a so far unknown active role for T cells finding their CNS target.

The first cell to be contacted by the circulating activated T cell is the postcapillary endothelium cell. Indeed, it is tempting to speculate that target specificity of infiltration is initiated at this first level of contact. A cell able to present antigen to the receptor of a T cell, must express Ia antigens. In fact, it has been reported that cultured endothelial cells of human origin can be induced to express Ia antigen [21], and that human endothelia can present antigen to autologous T cells [19]. In rodents, the situation is less clear.

The "simple" view of the endothelium acting as antigen-presenting cell is complicated by the fact that T line cells cross the BBB and cause disease only in their activated state. Small, resting T line cells are no longer able to transfer EAE. Since resting T cells recognize antigen perfectly well, it seems probable that crossing of the BBB by activated T blasts requires mechanisms other than immune recognition. In fact, recent experiments by Savion et al. [25] have shown that activated T cells are able to penetrate an endothelial monolayer in vitro and attack the underlying basal membrane. It should also be noted that penetration of endothelium is by no means exclusive to the target tissues in transferred autoimmune disease. Activated T cells have been shown to routinely pass through vessel walls in the lung, in the thymus, and in other lymphoid organs, and penetration of specialized venule endothelia is a physiologic process in the recirculation of migrant lymphocytes [10].

It is noteworthy that recently T cells have been observed in normal brain white matter using monoclonal antibodies and immunocytochemistry [4]. Whether such intruding T blasts are destined to remain occasional "passenger lymphocytes", or whether they constitute "routine patrols" which can be reactivated in the target tissue to proliferate, provided they find "their" antigen presented by local brain cells, is certainly a key to understand brain immune reactivity. Classical antigen-presenting cells like macrophages or dendritic cells are absent in the brain parenchyme, but recent experiments have shown that autoantigens could be presented by astrocytes on the peripheral side of the BBB. It has been found that astrocytes produce Interleukin 1 [8], a T cell coactivating factor, and that they can present antigen to T cells in vitro and thus enter in a complex reciprocal interaction with T cells which not only leads to an immunospecific activation of the T cells but on the other side to increased expression of Ia antigens on astrocytes [9]. (Discussed in more details by Fontana in this series.) It is thus conceivable that astrocytes with their tight mesh net of processes could activate the T cells which penetrated the endothelium and basal membrane in the perivascular cuff. Figure 1 gives an impression of how tightly lymphocytes adhere to the perivascular tissue, even after mechanical isolation of the brain vessels.

## Novel Experimental Therapies

Conventional immunosuppressive therapy of autoaggressive disease has been unsatisfactory. First, undesirable sequelae include depression of immune reactivity in infection and possibly also in protection against tumor growth. Also, at least some immunosuppressants can have a destructive effect on the hematopoietic system. Also finally, suboptimal dosage of immunosuppressants can lead to paradoxical exacerbations of the disease, which may well be due to a weakening of physiologic counterregulatory pathways.

An ideal immunotherapy of autoaggressive disease should be specific. It should affect only the autoaggressive lymphocyte clones, whereas all the other lymphocyte clones should be left unimpaired. Immunospecific strategies have been designed experimentally using both humoral as well as cellular agents. Among the humoral approaches, anti-idiotypic antibodies directed against the antigen-binding sites of autoantibodies or autoaggressive T cells have gained wide interest as well as humoral suppressor factors which are secreted either by autoreactive suppressor hybridomas or by suppressor lines. Another, semi-specific approach to manipulate autoimmune disease has been developed by Steinman et al. [28]. It has been known for some time that several autoimmune diseases are linked to the HLA system, especially to determinants of the HLA-D region. It appears that HLA-D region-encoded immune regulation genes (Ir genes) are directly responsible for T lymphocyte-mediated autoimmunity. The autoantigen is recognized by autoreactive T cells in the context of Ia antigens of the disease-prone HLA-D phenotype. Thus, since most humans are heterozygous in their HLA-D region, treatment with anti-Ia antibodies against the disease-associated Ia haplotype should preferentially interfere with the autoimmune reaction. All immune reactions depending on corecognition with this haplotype would be interfered with in addition, whereas the

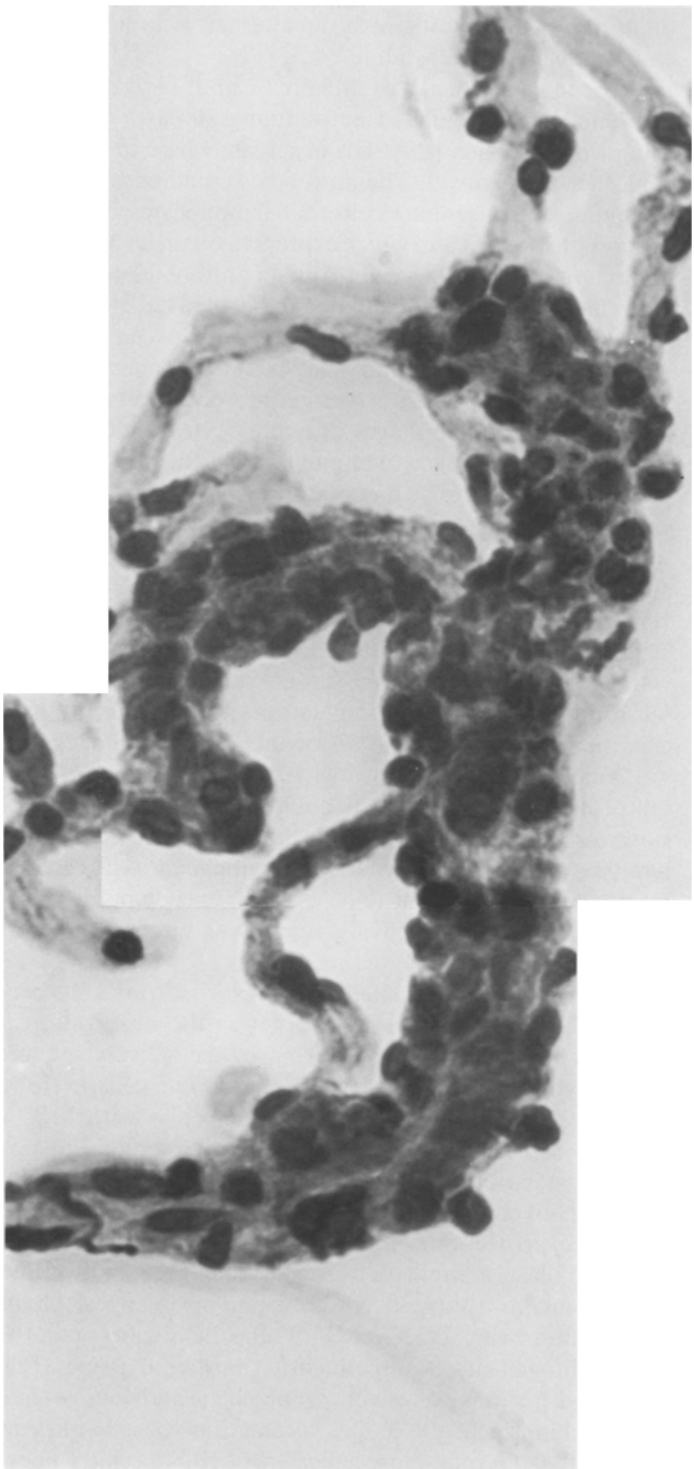

**Fig. 1.** Perivascular cuff isolated from the brain of a Lewis rat with severe T-cell line transferred EAE

other immune reactions with the alternative Ia haplotype as restricting elements would be left untouchend.

Recent experiments by Steinman and McDevitt suggest that a number of different Ir-gene regulated autoimmune diseases in rodents can be manipulated using this approach [28]. The exact site where in vivo applied anti-Ia antibodies could act is unknown. The antibodies could act at the primary stage of antigen presentation by presenter cells to T lymphocytes. They could furthermore bind to freshly activated T cells, which express Ia on their surface, and they could disturb the homing pattern of these cells. Anti-Ia antibodies could also bind to endothelial Ia molecules, which were postulated to be involved in T cell homing, and finally, anti-Ia could disturb the effector interaction between T cells and the CNS target. It remains to be established to what extent treatment in vivo with anti-Ia antibodies affects those cells which express either constitutive or inducible Ia antigen, e. g., dendritic cells, macrophages, B lymphocytes, endothelia, and epithelia.

A different approach to immunospecific therapy relies on the use of autoantigen-specific T cell lines. The fact that autoaggressive T lines had been selected from in vivo primed, and even unprimed normal rat lymphocyte populations strongly suggests, if not proves that the normal immune system contains potentially autoaggressive T cell clones as normal components. There must be mechanisms which under normal circumstances guarantee that the potentially autoaggressive T cells are not activated to attack the body's tissues. The most probable mechanisms warranting self-tolerance are specific suppressor pathways. In autoaggression, this self-protective suppressive regulation is pathologically reduced, and immunospecific therapy would aim to restor the self-tolerogenic equilibrium.

Principally, there are two ways to reach this goal. First, and most directly, the self-protective suppressor cells could be isolated, propagated in vitro, and re-infused suppressor cells would specifically strengthen suppression of the pathogenic lymphocyte clones. Alternatively, pathogenic self-reactive T line cells could be inactivated in a way that they lose their encephalitogenic capacity but retain their surface properties to activate suppressor mechanisms. This approach resembles vaccination with attenuated germs.

Application of attenuated autoaggressive T lines has been initiated by Ben-Nun et al. [2]. The basis for this work was the observation that Lewis rats, which spontaneously had recovered from either actively induced EAE, or from EAE transferred by activated MBP-specific T line cells, were resistant to subsequent immunization with encephalitogenic injection with MBP in CFA. This induced state of resistance was explained by induction of suppressive anti-idiotypic counterregulation.

Ben-Nun et al. used irradiated activated MBP-specific T line cells in an attempt to induce protection without preceding clinical disease. Indeed, irradiation of activated blasts completely abrogates their encephalitogenic potential and injected animals are to a large degree resistant to later encephalitogenic immunization. Vaccination with inactivated T line cells does not, however, protect from encephalitogenicity of subsequently transferred T lines. Furthermore, the effect of irradiated T cells is exclusively prophylactic and does not influence ongoing disease. The mechanism of the T line vaccination effect is unclear to date. It should be stressed that it is immunospecific. Only MBP-specific T lines will interfere with EAE

induction. Irradiated activated T line blasts of different specificity, in contrast, are ineffective. Recently, Holoshitz et al. [11] have established a different autoimmune T line model, which involves transfer of arthritis with rat T line cells specific for a determinant of mycobacteria. They found that one subclone which was ineffective in transferring disease to hosts, was fully capable of transferring protection to recipient rats. Another clone-transferred disease in the unirradiated activated state, but did not protect the hosts after irradiation. The molecular basis for this divergent behavior is unclear, its evaluation will, however, be of the highest interest for designing forthcoming therapeutic strategies. The alternative and more direct approach to immunospecific therapy with autoreactive T lines is the use of functionally suppressive T lymphocyte lines. This term is operational and implies that such T lines, in their activated state for some reason are unable to transfer clinical disease but actively interfere with induction or transfer of autoaggression, although the cells are fully specific for autoantigen.

We isolated a few years ago a MBP-specific T line, *bs*, from a BS strain rat. The BS strain has interesting similarities and differences as compared to the strain Lewis. Both strains share the key regions of their MHC (class I and class II antigens are identical). They differ, however, in a MHC-related sublocus which is apparently the correlate of the murine Qa locus. Although both strains are identical in their I region, BS is resistant to active EAE induction, which is in contrast to Lewis, the classical susceptible strain. *Bs* line cells can transfer a mild EAE to syngeneic BS rats. Lewis rats are, however, completely resistant. We found, moreover, that transfer of viable activated *bs* cells, which would transfer EAE to BS rats, protects Lewis rats from subsequent active immunization with MBP/CFA. This protective effect in so far resembles protection of Lewis rats with irradiated autoaggressive line cells. *Bs* cells protect, in addition, Lewis rats from supralethal doses of Lewis-derived encephalitogenic T line cells. Furthermore, *bs* line cells act not only prophylactically, but they are able to actively suppress the encephalitogenic effect of supralethal doses of Lewis line cells. A dose of $10 \times 10^6$ *bs* cells suppresses the lethal effect of $2 \times 10^6$ Lewis-derived L.BP cells. Finally, *bs* cells can influence the course of actively induced EAE by injection after immunization. *Bs* cells injected i. v. 4 days after immunization of Lewis rats with MBP/CFA delay the onset of disease by 3–4 days, and reduce the amplitude of clinical signs. To date, it has, however, not been possible to completely abrogate established clinical EAE.

As in the other therapeutic models, the exact therapeutic mechanism of the *bs* line cells is not understood. We know that the suppression is immunospecific and unique to line *bs*. *Bs* cells, which are specific for guinea pig myelin basic protein, protect Lewis rats from central nervous system localized EAE. They are, however, ineffective in peripheral nervous system EAN, which was transferred by T line cells specific for the P2 protein of peripheral nerve myelin. In addition, a BS strain-derived line specific for PPD does not protect from EAE, nor does another BS-derived MBP-specific T line, BS.BP. *Bs* and BS.BP both recognize MBP and transfer mild EAE to BS rats. They differ, however, in their fine specificity pattern. *Bs* cells recognize only intact guinea pig myelin basic protein, but surprisingly none of their protease cleavage products, nor rat basic protein. BS.BP behaves similar to the Lewis line L.BP, recognizing both intact GP-MBP, as well as the cleavage peptide P1, and less so P2 and P4. Finally, they can also be stimulated to a modest degree by

rat BP. It is not known at present whether and how this difference in fine specificity can be related to the protective effect of *bs*. It is, however, clear that protection is an active action of the injected cells. Irradiation before transfer completely abrogates the protective effect. Furthermore, activated *bs* blasts are much more effective than quiescent medium size line cells, which are kept for prolonged periods in growth factor medium. Certainly suppression of encephalitogenicity of L.BP cells by *bs* line cells is not due to direct suppressive interaction. When cultured in vitro, both cells coexist and react against antigen independently without interfering with each other. It should also be noted that both L.BP and *bs* lines do not differ in any phenotypic marker. Both express the W3/25 determinant and lack OX8, which qualifies them as either T helper cells, or as effector cells in delayed type hypersensitivity reactions.

## Conclusion

Although the effector mechanisms which lead to demyelination in autoimmune disease of the brain are still incompletely understood, several lines of evidence suggest that autoimmune myelin destruction occurs in several steps and that T cells play a pivotal role at least in the critical stage of autoantigen-specific inflammation. In the actively demyelinating lesions the predomiant cells of the mononuclear infiltrates are T lymphocytes. Furthermore, in contrast to the normal CNS which lacks Ia positive cells, inflammatory brain lesions contain a large number and a wide variety of Ia-bearing cells. Ia antigens, which are the product of the MHC-linked immune response genes, are a prerequisite for immunogenic antigen presentation to activate T cellular immune mechanisms. Ia antigens act at the molecular basis of the genetic control of autoimmune susceptibility and thus provide one target structure for manipulating autoimmune disease with monoclonal antibodies. The CNS cells which can express Ia have not been positively identified so far in vivo. The candidate cells include microglia and endothelial cells. It is remarkable that, in addition, astrocytes can be induced to express Ia in vitro, and are highly effective in presenting antigens to T cells. Whether the so-called "reactive" astrocytes in early demyelinating lesions have a similar function is an attractive possibility.

Demyelinating and autoimmune inflammatory diseases are transferable by T cells in animal models. The establishment of long-term T cell lines has paved the way for extensive studies of their pathogenetic role. The few years since T line technology has been available, have already brought new insights into problems like genetic control of CNS autoimmune disease and interactions between the autoaggressive lymphocytes with their target tissues. Current studies are focussed on the molecular basis of antigen fine specificity, antigen presentation, T cell homing, and target interaction.

An aspect with important clinical potential is the therapeutic application of T line technology, which has already been initiated in the past years by experimental "vaccination" against autoimmune diseases. Attenuated autoimmunogenic T line cells have been successfully used to protect animal from subsequent active disease induction. Potentially even more promising is the application of specifically suppressive autoreactive T cell lines which act not only porphylactically, but also have a direct therapeutic effect.

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