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IMMUNE REACTIVITY IN THE NERVOUS SYSTEM: MODULATION OF T-LYMPHOCYTE ACTIVATION BY GLIAL CELLS

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SUMMARY

The vertebrate central nervous system (CNS) has been traditionally thought to be inaccessible for the passenger lymphocytes of the immune system. This does not seem to be the case: activated T-lymphocytes can readily cross the endothelial blood–brain barrier (BBB) and some glial cells, notably the astrocytes, seem to be programmed to act as most efficient and complex partners for antigen-specific T-lymphocytes.

We used myelin basic protein (MBP) specific permanent rat T-lymphocyte lines as probes to assess the immune status of the CNS. These cells, upon activation *in vitro*, are able to transfer lethal, experimentally induced autoimmune-encephalomyelitis (EAE) to normal syngeneic recipients. Activated T-lymphocytes, but not resting ones, can break through the BBB irrespective of their antigen specificity. Immune surveillance of the CNS thus seems to be executed by activated T-lymphocytes.

Having crossed the BBB, the activated T-cells interact with local glial cells by releasing factors, including interferon-γ, which induce astrocytes to synthesize and express, on their membranes, class II major histocompatibility antigens (Ia determinants), which are critically required for immunogenic presentation of antigens to T-cells. Indeed, Ia-induced astrocytes of the CNS (and the Schwann cells of peripheral nerves) are efficient antigen presenter cells, which are able strongly to up-regulate antigen-reactive T-lymphocytes.

In addition, it has recently been shown that at least some astrocytes are able to down-regulate immune cells. Some, but not all, astrocytes are capable of suppressing activation of T-cells. This suppression can be modulated by interferon-γ, and is sensitive to irradiation. The question of whether suppression is mediated by direct cell-to-cell contact or *via* soluble mediators (e.g. apolipoprotein E) is under investigation. Astrocytes have been found to be most subtle regulators of immunocompetent T-cells. Most probably they are centrally involved in physiological immune reactivity of the CNS, and it will be tempting to learn how far glial cells are involved in transmitting regulatory signals between the immune and nervous systems.

Key words: astrocytes, T-lymphocytes, autoimmunity, myelin, blood–brain barrier.

INTRODUCTION

The brain, spinal cord and peripheral nervous system are the sites of functions that are the basis of human mind and behaviour. An organ system with such an elaborate function, would consequently be expected to have commensurately demanding housekeeping requirements. This seems indeed to be the case for the supply of nutritives, clearance of metabolites, structural support and compartmentalization, and ion equilibrium, to list just a few functions contributed by glial cells: the non-neural components of the nervous system.

Paradoxically, however, the tissues of the nervous system have been thought to be neglected by the surveillance mechanisms of the immune system. Lack of lymphatic drainage, the tight endothelial blood–brain barrier (BBB), lack of major histocompatibility complex (MHC) antigens and decreased graft rejection responses have been quoted in support of this concept (Wekerle, Linington, Lassman & Meyermann, 1986). In this article, a series of experiments will be discussed, the results of which suggest that the nervous system is not inaccessible to the immune system. An important conclusion will be, that glial cells, particularly astrocytes, have the capacity efficiently to interact with migrating immune cells. Astrocytes, are able not only to activate circulating T-lymphocytes but, in addition, efficiently to down-regulate these cells to form key elements at a regulator interface between the immune and nervous systems.

MYELIN-SPECIFIC T-LYMPHOCYTE LINES AS PROBES OF THE IMMUNE STATUS OF THE NERVOUS SYSTEM

One way to study directly the immune cell circulation within the CNS parenchyma, is to use permanent lines of T-lymphocytes that recognize monospecifically defined molecules on CNS structures.

T-lymphocytes, selected for reactivity against myelin basic protein (MBP) (a major structural protein of CNS myelin) are especially suitable immunological probes. First, it has been known for many years that immunization of inbred Lewis rats with MBP will lead to autoimmune inflammation of the CNS, experimental autoimmune encephalomyelitis (EAE) (Roboz-Einstein, Robertson, DiCaprio & Moore, 1962), and that this disease can be transferred to naive syngeneic recipient rats by immune lymphocytes, but not by humoral factors (Paterson, 1960). Second, myelin basic protein is an autoantigen which has been thoroughly characterized at molecular and cellular levels. Its sequence is known in several species (Eylar & Jackson, 1974) and its gene has recently been cloned (Roach *et al.* 1983). Third, the immune response against MBP is under the strict control of immune response (Ir) genes in the rat (Williams & Moore, 1973; Gasser, Palm & Gonatas, 1975; Günther, Odenthal & Wechsler, 1978) and in the mouse (Fritz *et al.* 1985).

MBP-specific T-lymphocyte lines have been established both from pre-immunized Lewis rats (Ben-Nun, Wekerle & Cohen, 1981) and from unprimed, normal Lewis lymph node cells (Schluesener & Wekerle, 1985). All Lewis rat T-lines selected for

reactivity against intact MBP share important properties. First, they all express a membrane phenotype which is typical for the T-helper subset of T-lymphocytes: they bind the monoclonal marker antibody W3/25, but exclude antibody OX8 (Mason *et al.* 1983). Then, all these T-lines are exclusively specific for 'their' selecting antigen, MBP. Furthermore, they recognize MBP in the molecular context of major histocompatibility complex class II antigens, Ia determinants (Fierz *et al.* 1985). Finally, T-lines, selected for reactivity against the intact MBP molecule, are not specific for rat epitopes that are randomly positioned on the MBP molecule. In the Lewis rat, all MBP-specific T-lines are reactive against one circumscript molecular epitope represented by amino acid sequence 66–68 (Sun & Wekerle, 1986). This epitope has been defined as the main encephalitogenic region for the Lewis rat (Kibler *et al.* 1977). In the SJL mouse, the encephalitogenic MBP region of the SJL strain is located within amino acid sequence 89–169, whereas PL mice preferentially respond to sequence 1–37 (Fritz *et al.* 1985). Thus, individual immune systems which are controlled by distinct Ir gene sets will recognize individual epitopes on a given myelin autoantigen.

Lewis rat-derived MBP-specific T-lines are not only *autoreactive*, but *autoaggressive*. *In vitro*, activated MBP-specific T-line cells are able to mediate violent, acute CNS-specific immune inflammation. Typical clinical and histological autoimmune encephalomyelitis (EAE) can be transferred to normal syngeneic recipient rats with cell doses as low as 1×10^4 cells/recipient (Schluesener & Wekerle, 1985). In these cases, EAE is relatively mild and the recipient will recover spontaneously. At higher doses, starting at $1–2\times10^6$ cells/recipient, the disease will take an unfailingly lethal course.

The course of transferred EAE is peculiar. Only after a symptom-free period of 3 days does the treated host develop limb paralysis, which is initially restricted to the tail and then ascends cranially *via* hind- and forelimbs. Usually, within 5–6 days, the encephalitic rat will fall into stupor and die. The extremely acute course of target tissue destruction has been followed semiquantitatively by electrophysiological methods in T-line-mediated experimental autoimmune neuritis (EAN), a system sharing its fundamentals with T-line-mediated EAE. In EAN, T-lines are selected for recognizing the P2 protein of peripheral nerve myelin (Linington *et al.* 1984). Like MBP, the P2 molecule is a basic protein located at the cytoplasmic face of the myelin membrane (Omlin, Webster, Palkovits & Cohen, 1982). Lewis rats respond against a defined molecular sequence, determined by Uyemura *et al.* (1982). In a manner similar to MBP-specific T-lines, injection of activated P2-specific T-cells does not lead to clinical symptoms within the first 3 days post-injection. Continuous monitoring of the transmitting function of sciatic nerves revealed that from day 3, nerve function was abruptly reduced; within 12 h all functions were lost. Complete recovery of these nerves took at least 10 weeks (Heininger *et al.* 1986). The electrophysiological development was faithfully paralleled by clinical paralysis (Linington *et al.* 1984) and the development of typical mononuclear infiltration (Izumo, Linington, Wekerle & Meyermann, 1985).

The fact that lethally autoaggressive T-lymphocyte lines can be isolated from completely normal immune cell populations has profound immunological implications. Assuming phenotypic stability of the recombined gene of the T-cell receptor variable region (Ikuda, Ogura, Shimizu & Honjo, 1985; Leiden & Strominger, 1986), one can conclude that the antigen receptor specificity of the T-line cells is identical with that of their precursors in the initial unselected lymphocyte population. This strongly supports the concept that the normal T-cell immune repertoire contains potentially autoaggressive T-cell clones as normal components. Consequently, suppressive regulation rather than physical elimination of autoaggressive T-lymphocyte clones should be the basis of immunological self-tolerance.

T-LINE CELLS ARE AN AUTONOMOUS PATHOGEN IN MEDIATING EAE

The histological lesion arising during T-line-mediated EAE cannot be distinguished from that seen in acute EAE induced by active immunization. In both models, the key morphological features are mononuclear infiltrations predominantly concentrated around post-capillary CNS vessels. In addition, some diffuse infiltration is seen in the adjacent areas (Vass, Lassman, Wekerle & Wisniewski, 1986). The second main feature of the EAE lesion is the activation of astrocytes. Demyelination (a hallmark of multiple sclerosis and chronic EAE) is, however, inconspicuous and restricted to areas close to acute infiltration (Lassmann, 1983). Immunocytochemistry has demonstrated that most, if not all, of the infiltrating lymphocytes are T-cells of the same subset as the injected T-lines. Nevertheless, transfers of isotope-prelabelled encephalitogenic T-line cells, and autoradiographic localization of the injected cells within the inflammatory lesions, revealed that only a small minority of the infiltrating T-cells were indeed the progeny of the injected pathogenic T-cells. The overwhelming majority, however, must have been contributed by the host immune system (Wekerle *et al.* 1986).

EAE has been thought to result from mechanisms involving delayed type hypersensitivity (DTH). These are based on complex interactions between immune cells of different subsets, on the one hand, and macrophages and other accessory cells on the other. Is the recruitment of host-derived inflammation thus essential for EAE development? We used lethally irradiated (750 R) Lewis rats as immunosuppressed T-line recipients to answer this question. These animals are severely depleted both of their own immune cells and of their haemopoietic elements, and unless restored with exogenous haemopoietic stem cells, will die within 2–3 weeks of anaemia or infections. In these experiments, lethally irradiated Lewis rats were injected with sublethal doses of MBP-specific T-line cells and were scored daily for clinical symptoms (Fig. 1). They were compared to unirradiated, control animals that had been treated with the same dose of activated T-line cells. Remarkably, both animal groups developed EAE and in both the course of the disease was practically identical. Like unirradiated controls, irradiated animals showed a lag phase of 3 days preceding onset of clinical symptoms. Thereafter, neurological signs developed abruptly in both groups. Most unexpectedly, however, the T-cell-mediated disease was self-

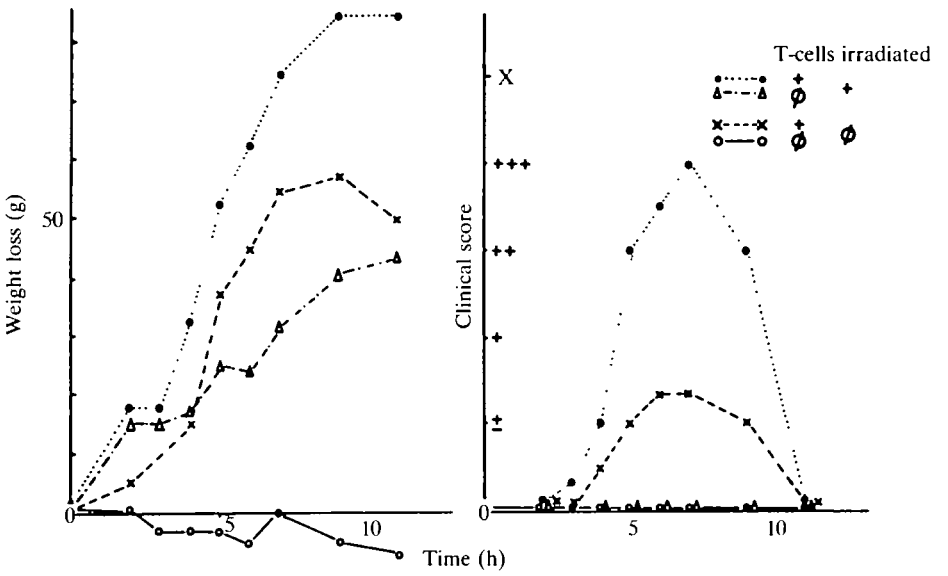

Fig. 1. MPB-specific T-line cells transfer EAE to normal and irradiated recipient rats. Clinical EAE was evaluated by determination of weight loss and clinical scoring: ±, loss of tail tonus; +, paralysis of hind limb; ++, tetraplegia; +++, stupor, convulsions; X, death.

limiting, even in irradiated animals. All animals recovered from neurological symptoms by day 11.

Irradiated rats receiving lethal doses of MBP-specific T-cells showed morphological lesions with interesting histological patterns. In contrast to the CNS lesions of unirradiated control animals, EAE lesions in the spinal cords of irradiated rats lacked completely the dense perivascular infiltrations. Instead, scattered mononuclear cells, presumably activated T-lymphocytes, infiltrated the adjacent parenchyma as when EAE was transferred by primed T-cells into irradiated rats (Sedgwick, Brostoff & Mason, 1987) (Fig. 2). Also, in contrast to the normally simplified pattern of cell infiltration, there was a severe defect of the BBB as reflected by multiple focal bleedings through CNS blood vessel walls. These and severe oedema are the main morphological characteristics of severe clinical changes.

Development of T-line-mediated EAE thus does not depend on the dense perivascular and parenchymal infiltrations by host-derived cells. In contrast, it appears that the encephalitogenic T-line cells are autonomous in causing the CNS defects of EAE. We assume that these defects are caused by direct cytotoxic interactions with local glial cells (Sun & Wekerle, 1986). Moreover, these experiments indicate that migration of host-derived 'suppressor cells' into EAE lesions, which occurs predominantly in the late phases of the disease (Hickey & Gonatas, 1984), is not critical in terminating EAE. As will be shown below – as an interesting alternative – local glial cells may be involved in down-regulation of the local T-cell response.

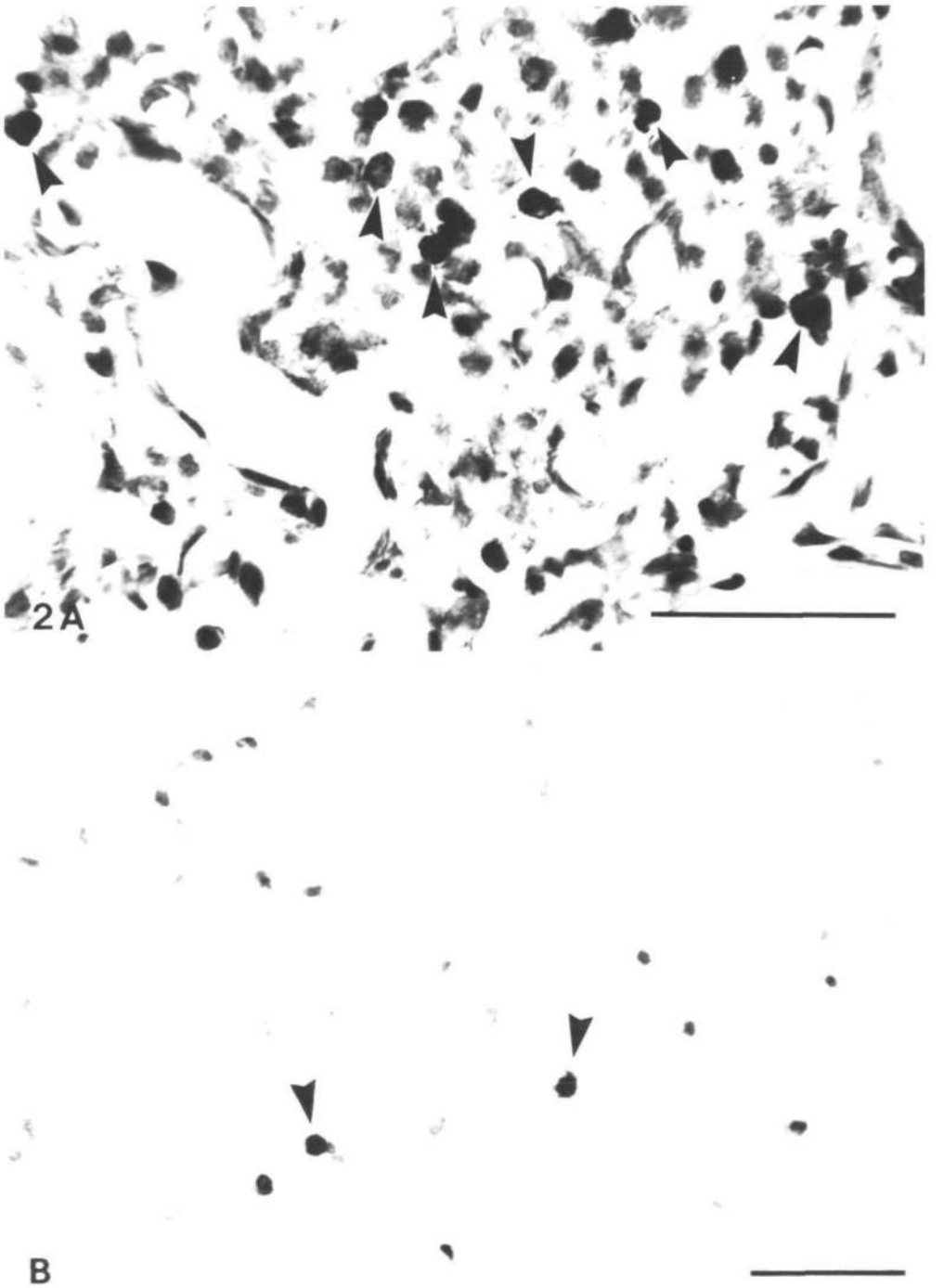

Fig. 2. Immune cell infiltrate in T-line-mediated EAE of a lethally irradiated recipient rat. Scattered activated T-lymphocytes bind monoclonal antibody ART-18, an activation marker recognizing the Il-2 receptor (B). The recipient's spleen is depleted of lymphoid cells, except for some scattered lymphoblasts, presumably T-line progeny (arrowhead). Scale bar, 40 μm.

MIGRATION OF MYELIN-SPECIFIC T-LYMPHOCYTES TO THE CENTRAL NERVOUS SYSTEM

One of the most impressive aspects of T-line-mediated autoimmune disease is its tissue specificity. MBP-specific T-lines transfer an autoimmune disease which is exclusively restricted to the CNS, whereas inflammation mediated by P2-specific T-lines affects the peripheral nervous system (PNS) but not adjacent CNS tissues (Izumo *et al.* 1985; Wekerle *et al.* 1986). Since in transferred EAE the pathogenic T-lymphocytes are injected intravenously and subsequently circulate through the blood vascular system, the BBB endothelium, which separates the circulation from the CNS parenchyma, can be expected to play a critical role in target retrieval by the encephalitogenic T-cells. CNS endothelia could thus present MBP molecules (in the context of Ia determinants) on their luminal surface, and these autoimmunogenic molecular complexes could act as a target signal for circulating T-line cells. Endothelial Ia has been reported by several groups (Traugott, McFarlin & Raine, 1986; Sobel, Natale & Schneeberger, 1987), although others (Matsumoto, Hara, Tanaka & Fujiwara, 1986; Vass *et al.* 1986) have been unable to confirm this (Fig. 3).

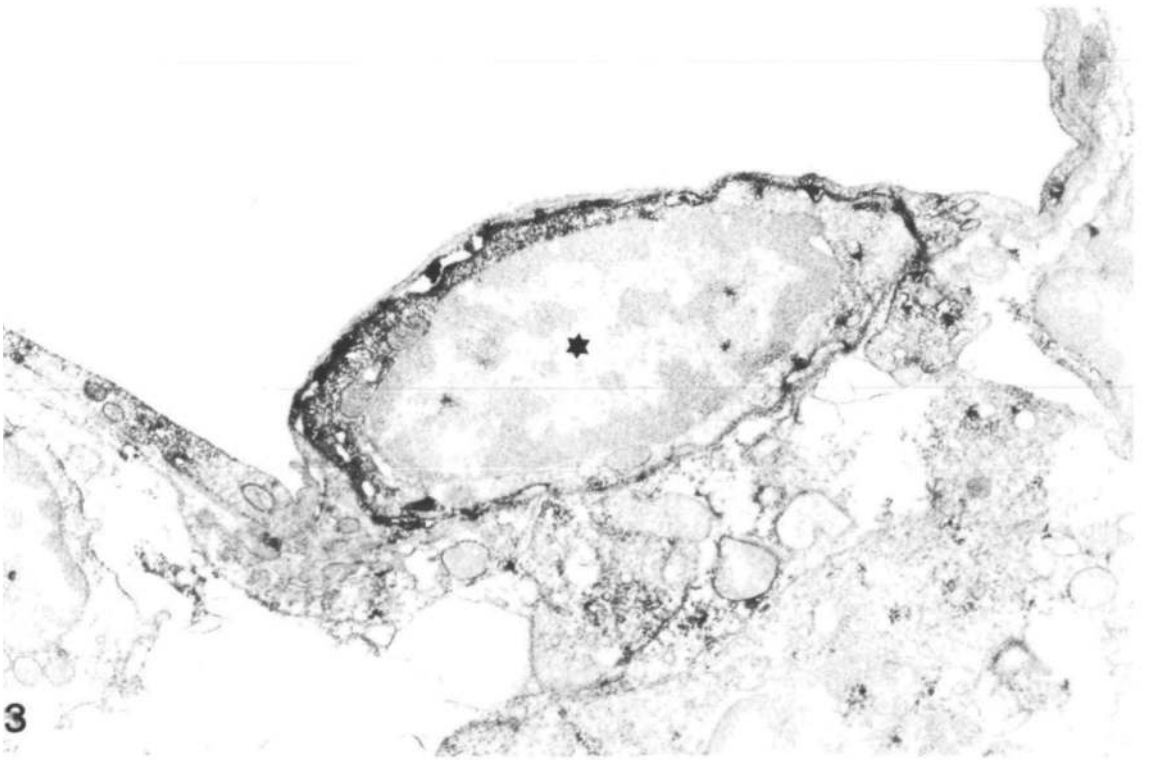

Fig. 3. Ia determinants associated with the blood–brain barrier in T-line-mediated EAE. An activated, Ia-positive lymphoblast (asterisk) beneath negative CNS endothelium. Immunocytochemical electron microscopy of Lewis rat spinal cord. Ultra-thin section, 5 days after injection of encephalitogenic T-line cells. (OX6) monoclonal anti-Ia antibody plus avidin/biotin/peroxidase-complex. Magnification, ×20 000.

In vitro assays have shown that cells from CNS microvessel preparations have the capacity to present antigen to primed T-cell populations (McCarron, Kempski, Spatz & McFarlin, 1985), whereas pure, clonal populations of rat CNS endothelia were completely inert (H. Wekerle & W. Risau, unpublished results).

There seems to be an alternative, antigen-independent pathway, leading T-lymphocytes through the BBB. Activated, but not resting T-cells, have the capacity to break through CNS endothelia and to invade the parenchyma irrespective of their antigen specificity.

It has been known for years that T-line cells mediate EAE with stunning efficiency, provided they are preactivated *in vitro* (Schluesener & Wekerle, 1985). In contrast, resting MBP-specific T-line cells will not transfer disease, even if injected in extremely high doses (Naparstek *et al.* 1983). Activation-dependent, but antigen-independent, BBB transmigration by T-lymphocytes was formally documented using [^{14}C]thymidine-labelled T-line cells of different antigenic specificities and by their subsequent autoradiographic localization in CNS tissues. We found that a small but constant number of MBP-specific activated T-lymphoblasts appeared in the brain parenchyma within 24 h post-injection. During the following 48 h their proportion remained roughly constant. At the beginning of neurological symptoms (approx. 3 days post-injection) a second, massive wave of cellular invasion occurred. This led to the immigration of millions of unlabelled host-recruited immune cells. Surprisingly, the first (but not the second) peak of invasion was also seen after injection of isotope-labelled, activated T-line cells which were specific for ovalbumin (a model antigen for foreign determinants which do not occur naturally within the host nervous system). Again, labelled ovalbumin-specific T-cells were demonstrable by 24 h post-injection (Fig. 4). These cells were diluted out during the following few days without any subsequent second invasion peak.

Activated, but not resting, T-lymphocytes, thus seem to have access to the CNS parenchyma, irrespective of their antigen specificity. There are interesting parallels between migration of normal T-lymphoblasts and the behaviour of brain-metastasizing tumour cells (Nicolson, 1984). In all these examples, migration through the BBB seems to involve a similar, stepwise mechanism. First, the cells must attach to the luminal side of the BBB endothelium. Then, due to a shared pattern of induced and secreted proteolytic and glycolytic enzymes, the elaborate system of intercellular tight junctions opens (Nagy, Peters & Hüttner, 1984) and, finally, the cells pass through the surrounding basal membranes (Naparstek, Cohen, Fuks & Vlodavsky, 1984). As will be discussed below, this migration pathway may be the basis for the specialized version of immune surveillance in the CNS.

ACTIVATION OF T-LYMPHOCYTES BY ANTIGEN-PRESENTING ASTROCYTES

If T-cells on their way to the CNS parenchyma do not identify their target organ at the endothelial level, the signal of target specificity must be at some stage *beyond* the BBB. Assuming that this signal depends on immunogenic presentation and

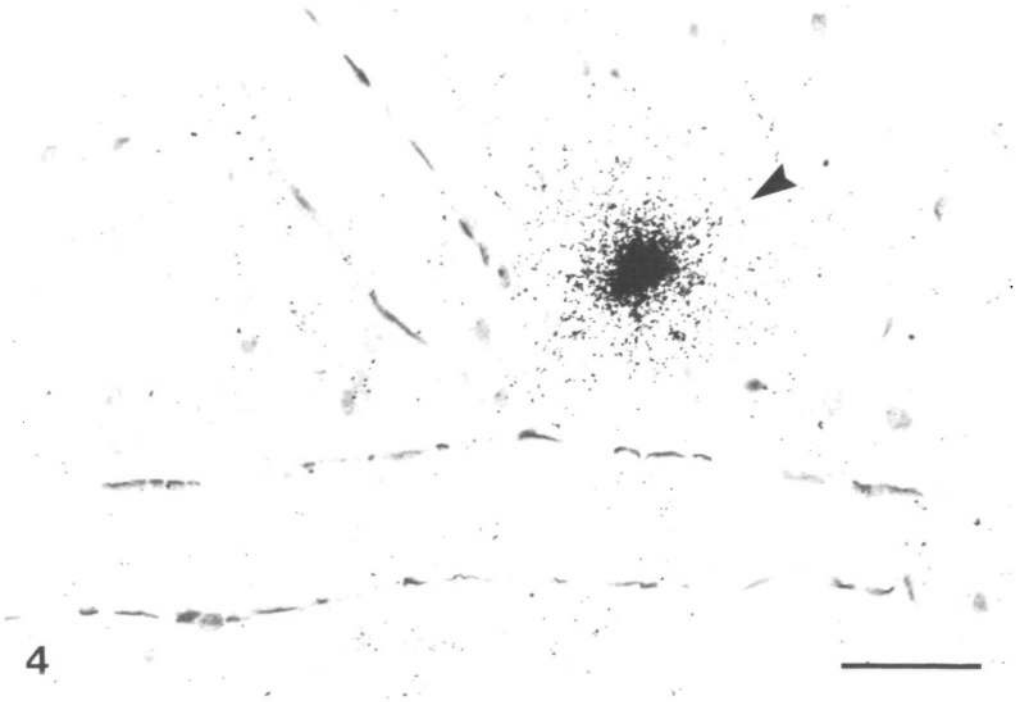

Fig. 4. Ovalbumin-specific activated T-line cell, within CNS parenchyma, 24 h post-injection. T-line cells were activated *in vitro*, prelabelled with [^{14}C]thymidine, and injected intravenously into a syngeneic rat. Radioactive cells were identified by thick-layer autoradiography. Scale bar, 40 μm.

recognition of the relevant (myelin) autoantigen, the normal CNS should contain, contrary to more prevailing beliefs, antigen-presenting cells (APC).

Astrocytes are likely candidates for more than one reason. First, one subset of astrocytes is tightly arranged around CNS blood vessels (Janzer & Raff, 1987), an ideal site for a strategically positioned APC, and second, astrocytes have been shown to interact intimately with immune cells. It has been claimed, for example, that they *respond* to several immune mediators, including interleukin 1 (Giulian & Lachman, 1985), and also that they can be induced to *produce* such factors (Fontana *et al.* 1982).

Astrocytes were tested for their potential antigen-presenting capacity by using purified astroglial cultures derived from perinatal Lewis rat brain. When such astrocyte monolayers were cultured together with syngeneic antigen-specific T-line cells, no conspicuous interactions were noted. However, immediately after addition of the relevant soluble antigen (MBP or ovalbumin) the specific T-cells aggregated around the astrocytes, and within 24 h they were transformed to enlarged, strongly proliferating lymphoblasts (Fontana, Fierz & Wekerle, 1984). Detailed analyses have shown that T-line cell activation was the consequence of immunospecific presentation and recognition of antigen. The reaction depended on the dose of antigen added, and antigen recognition was restricted by Ia determinants of the MHC.

Ia-restricted interactions were difficult to reconcile with the established observation that CNS parenchymal cells express little, if any, class I (Schachner & Sidman, 1973) and no class II MHC antigen (Hart & Fabre, 1981). Indeed, our cultured astrocytes were also negative for Ia antigens before presentation. However, when stained after interaction with lymphocytes, they had acquired Ia in considerable amounts (Fierz *et al.* 1985). Astrocytes, and a number of other cell types outside the nervous system, can be induced to synthesize and express Ia determinants either by mere contact with activated T-lymphocytes, as in our experiments (Fontana *et al.* 1984), or by T-lymphoblast-derived mediators such as interferon-γ (Hirsch, Wietzerbin, Pierres & Goridis, 1983; Wong *et al.* 1985). These interactions not only induce Ia expression on the surface, but unlike many other cells, enable the astrocytes to take up, process and immunogenically present soluble antigen. Astrocytes thus qualify as inducible, facultative APC.

DOWN-REGULATION OF ACTIVATED T-LYMPHOCYTES BY ASTROCYTES

The finding that EAE (transferred by encephalitogenic T-lines to irradiated recipients) produced a monophasic, self-limiting course of the disease, suggested the existence of counter-regulatory mechanisms acting on the pathogenic T-lymphocytes which were independent of the host immune system. Local glial cells could also be involved in these interactions. Indeed, screening a panel of long-term, cultured and cloned astrocyte lines revealed immunosuppressive activity. These clones were derived from a standard astrocyte culture isolated from neonatal Lewis rat brain. After 8 months of bulk culture, the $GFAP^+$ astrocytes were dissociated and cloned by limiting dilution techniques. All of these astrocyte sublines expressed GFAP in varying intensity and were inducible to express Ia. When screened for their capacity immunogenically to present auto- or foreign antigens to relevant T-line cells, the clones differed strongly in their APC capacity. This was documented most clearly in dose–response experiments using graded numbers of antigen-presenting astrocytes, with constant numbers of responding T-lymphocytes. Some clones were highly efficient at all cell densities, others presented antigen only at low cell densities, whereas a third group was completely ineffective. The incompetence of the latter to present antigen was not due to lack of Ia antigens on their surface, or to a defect in their capacity to present antigen, but rather reflected active suppression of T-cell activation. Non-reactive astrocyte clones, in contrast to antigen-presenting ones, were found strongly to suppress conventional T-cell activation by 'normal' APC (macrophages or dendritic cells from immune organs) plus antigen (Fig. 5). Preliminary evidence suggests that the suppressive activity is released into the culture supernatants. The molecular nature of the putative mediator is, however, unknown. Since these suppressive clones are fully active in the presence of indomethacin, prostaglandins do not seem to be involved in suppression. Possible candidates for suppressing activity are, however, either apolipoprotein E, which has been shown to be released by astrocytes upon interferon-γ pretreatment (Fig. 6:

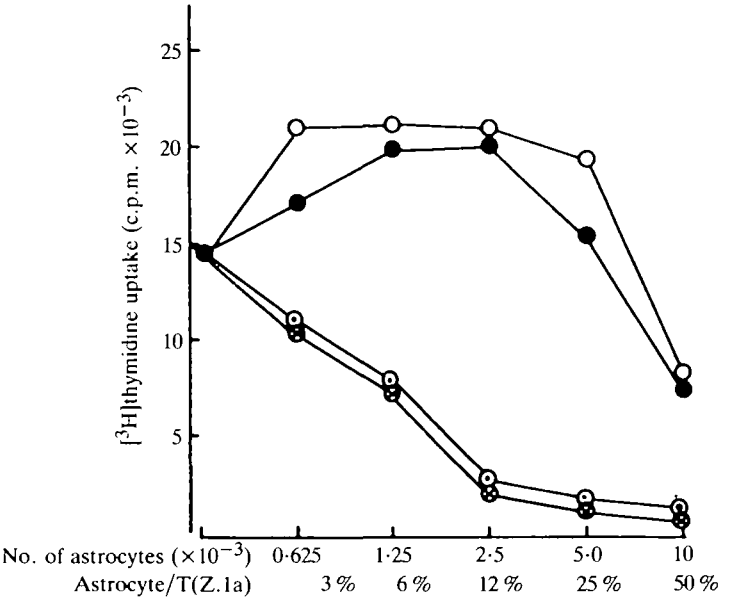

Fig. 5. Down-regulatory effect of individual astrocyte clones. MBP-specific T-line cells (Z.1a) were activated *in vitro* by syngeneic thymus cells (a standard source of 'professional' APC) presenting MBP. Astrocytic clone cells were added in graded numbers to evaluate their down-regulatory potential. Clones B5 (⊙) and B10 (⊗) were down-regulatory, whereas F10 (○) and C12 (●) were not.

Oropeza, Wekerle & Werb, 1987) or another, unrelated, astrocyte-derived suppressor factor (Schwyzer & Fontana, 1985).

IMPLICATIONS

Our findings that activated T-lymphocytes can cross the BBB and that astrocytes can be induced to up- and down-regulate the activity of intra-CNS T-lymphocytes have important implications. We have proposed that these observations reflect a specialized mode of immune surveillance in the CNS, which may have developed as an adaptation to the specific requirements of the vulnerable CNS tissues. Potential 'bystander damage' inherent to immune surveillance reactions is minimized by reducing the number of patrolling lymphocytes to the few activated T-cells in the blood circulation, and by focusing their response to perivascular areas. Moreover, the fact that only inducible APC can present antigen in the normal CNS may serve strictly to regulate and limit cellular immune responses (Wekerle *et al.* 1986).

The validity of this concept can be tested by examining its implicit predictions. Brain grafts, for example, should be accessible to activated, but not resting T-lymphocytes. This is indeed the case, for tissue transplants are promptly rejected after peripheral immunization with grafts of the same specificity (Head & Griffin, 1985). Also in graft-*vs*-host disease, T-cells have also been shown to invade CNS tissues (Hickey & Kimura, 1987). In addition, small, but significant, numbers of lymphocytes should be found in normal CNS tissue, and these T-cells should be

in a (post-)activated state: predictions which have been confirmed immunocytochemistry and the use of appropriate lymphocyte markers (Booss, Esiri, Tourtellotte & Mason, 1983; Hafler *et al.* 1985). Furthermore, astrocytes, having interacted with (auto-)immune T-cells, should show at least some Ia antigens on their surface, as has been demonstrated in several cases of myelin-specific autoimmune diseases (Traugott *et al.* 1986; Hickey, Osborn & Kirby, 1985). The fact that macrophages and/or microglial cells are normally more intensively stained in Ia immunocytochemistry

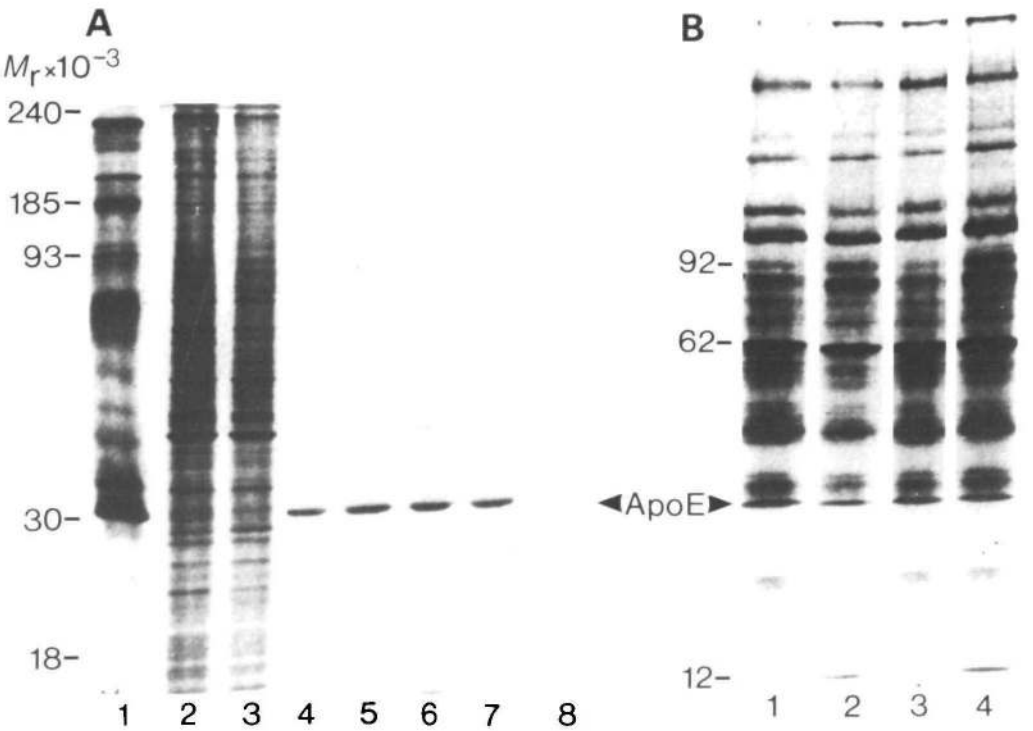

Fig. 6. Sodium dodecyl sulphate polyacrylamide gel electrophoresis of apolipoprotein E (ApoE) synthesized and secreted by astrocytes. (A) Confluent cultures of astrocytes (5×10^5/well) treated with various concentrations of IFN-γ in culture, for 48 h, then pulse-labelled with [^{35}S]methionine for 20 min. Total acid-precipitable labelled proteins and proteins immunoprecipitated from cell lysates with anti-ApoE IgG. Lane 1, proteins secreted by resident peritoneal macrophages. Lane 2, proteins in lysates of untreated astrocytes. Lane 3, proteins in lysates of astrocytes treated with IFN-γ (300 units ml^{-1}). Lanes 4–7, ApoE immunoprecipitated from lysates of untreated astrocytes. Lane 4, astrocytes treated with IFN-γ at the following concentrations: lane 5, 3 units ml^{-1}; lane 6, 30 units ml^{-1}; lane 7, 300 units ml^{-1}. Lane 8, proteins immunoprecipitated with anti-bovine serum albumin from lysates of astrocytes treated with IFN-γ (30 units ml^{-1}). (B) Confluent cultures of astrocytes (5×10^5/well) were treated with IFN-γ (30 units ml^{-1}), LPS (1 ng ml^{-1}) or both for 48 h in Dulbecco's modified Eagle's medium plus 10 % foetal calf serum, then labelled with [^{35}S]methionine for 2 h. Proteins secreted by untreated astrocytes (lane 1); lane 2, astrocytes treated with IFN-γ; lane 3, astrocytes treated with LPS; lane 4, astrocytes treated simultaneously with IFN-γ and LPS. Relative molecular mass markers and migration of ApoE are indicated (from Oropeza, Wekerle & Werb, 1987).

(Vass *et al.* 1986) supports the existence of marked counter-regulatory properties of astrocytes, as demonstrated in our present experiments.

Several fascinating, but so far unanswered, questions arise from the present observations. Apart from their role in immunosurveillance of the CNS, astrocytes that interact with CNS-patrolling lymphocytes could have physiological roles in modulating immune reactivity. After contacts with astrocytes, T-cells may 'export' CNS-derived stimuli to the peripheral immune system. Conversely, astrocyte may be modulated in their activity by contacts with local T-cells. It has been shown repeatedly that astrocytes respond to immune mediators (Fontana *et al.* 1982; Giulian & Lachman, 1985; Hirsch *et al.* 1983; Wong *et al.* 1985). Since our knowledge of the astrocyte function within the CNS is limited, the present interpretations of lympho–glial interactions remain speculative. Could human behaviour be conditioned by the immune status *via* the T-cell/astrocyte junction? Could general responses like alertness/sleep, temperature and muscular tonus be influenced by such mechanisms? More detailed information on lymphocyte/glia interactions will help to elucidate the pathogenesis of diseases, which so far have been enigmatic. Narcolepsy – a sleep disorder with a strikingly close association with immune response genes – (Seignalet, 1986) may be one of them.

There is no conclusive answer to these questions at present, but we are confident that an experimental approach to these problems will emerge.

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