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Review

A revised view of the central nervous system microenvironment and major histocompatibility complex class II antigen presentation

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Abstract

There are numerous observations reporting that phagocytes expressing major histocompatibility complex (MHC) Class II molecules are associated with the central nervous system (CNS) in normal and pathological conditions. Although MHC Class II expression is necessary for antigen presentation to CD4 + T-cells, it is not sufficient and co-stimulatory molecules are also required. We review here recent in vivo studies demonstrating that the microglia and perivascular macrophages are unable to initiate a primary immune response in the CNS microenvironment, but may support secondary immune responses. Although in vitro studies show that microglia do not support a primary immune response leading to T-cell proliferation, they do show that microglia may protect the CNS from the unwanted attentions of autoreactive T-cells by inducing their apoptosis. The lack of cells in the CNS parenchyma with the ability to initiate a primary immune response has a cost, namely that pathogens may persist in the CNS undetected by the immune system.

Keywords: Central nervous system; MHC class II; T-cell

1. Introduction

Antigen presentation is a remarkable process. A cell with this capacity ingests a foreign protein, partially digests it, exports peptides to the cell surface in association with major histocompatibility complex (MHC) molecules, and engages in binding to a T-cell. This process and the many underlying molecular interactions have been recently reviewed (Pieters, 1997; Watts, 1997). This article will focus on antigen presentation by MHC Class II bearing cells to CD4 T-cells in the central nervous system (CNS). It is important to recognise in this context that there are antigen presenting cells (APCs) with the capacity to initiate a primary immune response by the presentation of antigen to naive T-cells, and APCs that will stimulate a secondary response by the presentation of antigen to T-cells that have already been exposed to the antigen. It is only the dendritic cells (DCs) that have the former capacity, while a number of different cell types may be engaged in secondary responses (Steinman, 1991). Proliferation of the T-cell, the most commonly used assay for evidence of APC function, is one outcome of the antigen presentation process and depends on signals from co-stimulatory molecules other than those derived from engagement the T-cell receptor (TCR) with MHC Class II. However, as we shall review, TCR–MHC engagement that does produce T-cell proliferation, but does lead to T-cell cytokine production, is an important form of antigen presentation, as is TCR–MHC engagement that leads to neither but induces T-cell death.

The capacity of a cell to present antigen is a functional phenotype. Although the molecular profile of cells involved in antigen presentation is becoming increasingly better defined, and thus these cells can be visualised in tissue sections, in the final analysis antigen presentation can only be demonstrated functionally. The demonstration of this functional phenotype has commonly relied on the isolation of selected cell types from tissues, and in vitro assays are then used to demonstrate their functional capacity. This is a complex procedure given the many cell types to be found in any tissue, and the very potent antigen presenting capacity of some cells. Contamination of a cell population with a small percentage of professional APCs can significantly influence the response observed. Isolation of discreet purified populations of cells from tissue is no less complex when it comes to the CNS. In recent years,

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however, a number of studies both in vitro and in vivo have addressed the issue of antigen presentation in the CNS in novel ways. These studies are likely to have a significant impact on our understanding of diseases of the CNS with an immune component. Furthermore, as tissue transplantation and gene therapy become a clinical reality for the treatment of neurological disease, so the immunological problems associated with these treatments come more sharply into focus. We first review the phagocyte populations that have been identified in the different CNS compartments and express MHC Class II, a necessary, but not sufficient, criterion for antigen presentation.

2. Mononuclear phagocytes in CNS compartments

2.1. Microglia

The largest population of phagocytes associated with the CNS is the microglia. It is now widely accepted that these cells are of the mononuclear phagocyte lineage (Hickey and Kimura, 1988; Perry and Gordon, 1991; Kreutzberg, 1996). Cells of the mononuclear phagocyte lineage enter the developing nervous system during embryogenesis, and these macrophages are involved in the removal of cells undergoing apoptosis as a normal component of brain development. During brain maturation the macrophages within the parenchyma adopt a highly differentiated morphology and phenotype (Perry et al., 1985). The microglia represent about 10% of the non-neuronal cells in the CNS parenchyma (Lawson et al., 1990). As has been discussed elsewhere, these cells do not express a number of plasma membrane or cytoplasmic molecules found in association with other tissue macrophages and in general appear to have a quiescent or downregulated phenotype (Perry and Gordon, 1991; Kreutzberg, 1996). Of particular note in the present context is that microglia in the brains of healthy, young, adult rodents of many laboratory strains do not express immunohistochemically detectable levels of MHC Class II. The absence of MHC Class II on the microglia cannot be attributed to the immunohistochemical reagents or detection systems, since this antigen is readily detected in other populations of CNS macrophages (see below).

The health and age of the animals deserve comment. Microglia in aged but pathogen free animals have enhanced levels of MHC Class II (Perry et al., 1993), and microglia in animals with systemic infections also show enhanced levels of MHC Class II expression (Perry and Matyszak, unpublished observations). This situation contrasts with that seen in brain tissue taken from man, in which microglia are commonly reported as expressing MHC Class II (Hayes et al., 1987; McGeer et al., 1993). Of course, in contrast to rodents, humans do not generally live in SPF conditions. Although microglia do not constitutively expressed MHC Class II, it is readily upregulated by many forms of CNS pathology, indeed in the rat this has been the focus of a very diverse range of insults, Wallerian degeneration (Rao and Lund, 1989), spreading depression (Gehrmann et al., 1993), excitotoxic induced neurodegeneration (Akiyama et al., 1988), ischemic injury (Gehrmann et al., 1992), and during experimental allergic encephalomyelitis (EAE) (Vass et al., 1986). It has been suggested on the basis of this type of observation that microglia may thus be involved in antigen presentation, but it is now clear that this extrapolation is not always valid (see below). It is important to note that while MHC Class II is upregulated in the rat brain following diverse forms of injury, and also on the microglia from the diseased human brain, it is not the case for all species. In the mouse, MHC Class II expression is much less easily induced on microglia (Andersson et al., 1991; Lawson et al., 1994), and this may be of import for those engaged in studies using transgenic animals for the analysis of aspects of CNS pathology.

2.2. Perivascular and other CNS macrophages

There are a number of compartments associated with the CNS where cells which constitutively express MHC Class II are to be found; the perivascular space, the meninges and within the stroma of the choroid plexus. The perivascular macrophages have been considered a potentially important cell in the context of antigen presentation, since they are likely to be the first cell that a T-cell will encounter when it crosses the cerebral endothelium. It has been demonstrated that activated T-cells have the capacity to cross the intact blood-brain barrier (BBB), and this traffic may be an important component of the surveillance of the brain by the immune system (Wekerle et al., 1986). The perivascular macrophages also have a distinct phenotype, they express the ED-2 antigen (Graeber et al., 1989) and a percentage of them constitutively express MHC Class II, which is upregulated in reaction to injury (Streit et al., 1989). Apart from their potential role in immune surveillance of the CNS, these cells express the scavenger receptor and are actively phagocytic (Mato et al., 1996). These cells have been demonstrated to turnover somewhat more rapidly than microglia being replaced from the bone marrow within a period of a few months (Hickey et al., 1992). It is worth noting that the monocyte must cross an intact BBB to take up its position in the perivascular space. The fate of the perivascular cells that are replaced is not known. Whether they have the capacity to migrate back to lymphoid organs is not known and remains an important question.

MHC Class II expression is readily detected on cells in the meninges and within the stroma of the choroid plexus. These cells express a readily detectable spectrum of antigens expressed by other tissue macrophages, for example, MHC Class I, the leucocyte common antigen, CR3, F4/80 (Matyszak et al., 1992). These cells show evidence of

ongoing phagocytic activity. The macrophages in the meninges are a relatively radiation sensitive population cells and are replaced in a matter of days in radiation chimeras (Hickey et al., 1992).

2.3. Dendritic cells

The very low levels or virtual absence of MHC Class II from the CNS parenchyma (Hart and Fabre, 1981) precludes the presence of DCs, but it is of interest to know whether DCs are present in other compartments. In recent years, a number of reagents have been developed which recognise molecules relatively restricted to dendritic cells. The monoclonal antibody OX62 was raised against dendritic cells purified from lymph and recognises an integrin α_{E2} chain present on dendritic cells and γδ T-cells in the rat (Brenan and Puklavec, 1992; Brenan and Rees, 1997). Using this reagent, a small number of dendritic cells have been demonstrated to be present in the meninges and stroma of the choroid plexus of rats (Matyszak and Perry, 1996b). In the mouse, the monoclonal antibody NLDC145, also with a specificity for dendritic cells (Agger et al., 1990) labels a small population of cells in the stroma of the choroid plexus. There are no reports that cells bearing these antigens are to be found within the parenchyma or perivascular space of the normal CNS. Why this should be the case when they readily enter the parenchyma in pathological conditions (see below) is not clear.

MHC Class II expression is necessary, but not sufficient for effective antigen presentation to CD4 T-cells, and it is clear that professional APCs such as the dendritic cells express other molecules that co-operate in the intercellular adhesion and presentation process (Steinman, 1991). Although ICAM-1 and B-7 have been identified on microglia from man and mouse (Williams et al., 1994; Carson et al., 1998), but not rat (Ford et al., 1996), it is the functional assay that will carry the case forward to identify a cell as an antigen presenting cell.

3. Regulation of MHC Class II expression on the microglia

3.1. Normal microglia

The regulation of the microglia phenotype, their morphology and the expression of some antigens, has been investigated in vivo and in vitro. In vivo studies have drawn attention to the fact that the exclusion of plasma proteins plays some part in regulating the expression of cell surface antigens (Perry et al., 1992). The presence of serum has also been shown to reduce the formation of processes by microglia in vitro (Giulian et al., 1995). The serum factors involved in these processes are not known, although the astrocyte is an obvious cell type likely to be involved in the regulation of microglia phenotype. Giulian et al. (1995) report that in co-cultures experiments, astrocytes promote and maintain the morphological differentiation of microglia, derived from neonatal brain, but apparently have no effect on the morphological differentiation of other macrophage populations isolated from newborn rats. In contrast, Sievers et al. (1994) have shown that astrocytes or astrocyte conditioned medium has a potent effect not only on the differentiation of microglia derived from newborn rat brain, but also other macrophage populations. The astrocytes induced both a microglia morphology and the expression of ion channels believed to be typical of microglia following co-culture for more than seven days. Unfortunately in these and other in vitro studies investigating microglia phenotypic behaviour (Chamak and Mallat, 1991; Suzumura et al., 1991), the levels of expression of MHC antigens were not studied.

The factors which regulate MHC Class II expression on microglia are poorly understood, and indeed are poorly investigated. In the normal brain, the low levels of MHC Class II expression are likely to result from both a lack of inducing stimuli and the presence of downregulating stimuli. In vivo, the microglia are the most sensitive cells in the CNS to γ-interferon since systemic delivery (Steiniger and van der Meide, 1988) or intracranial injection of this cytokine (Vass and Lassmann, 1990) upregulates MHC expression by microglia more readily than other cells. There is, however, no endogenous expression of γ-interferon within the CNS. The factors that result in the downregulation of MHC Class II possibly involve both cell contact and soluble mediators. It has been demonstrated that macrophages in brain slice preparations express higher levels of MHC Class II in regions where there is tissue damage, while in the regions of intact neurons MHC Class II expression is lower (Neumann et al., 1996). When the spontaneous neuronal activity in these slices was silenced with the sodium channel blocker tetrodotoxin, MHC Class II was upregulated. Interestingly, astrocytes were also more readily induced to express MHC Class II by γ-interferon in this condition. This data suggests that local ion fluxes, or the secretion of neurotransmitters or prostaglandins, for example, may be involved in the regulation. However, it has recently been demonstrated in an in vitro adhesion assay that macrophages preferentially bind to neurones (Brown and Perry, 1998), possibly via a novel adhesion molecule (Brown and Perry, in preparation). The substrate to which a macrophage binds is known to have a potent effect on its state of activation and phenotype, and the unusual extracellular matrix of the CNS microenvironment may have important effects in this regard. Other molecules known to be expressed in the CNS include macrophage colony stimulating factor-1 (CSF-1) (Lodge and Sriram, 1996) which will downregulate expression of MHC Class II as will IL-10 (Frei et al., 1994). The combination of adhesion molecules and soluble factors that regulate the unusual phenotype of the microglia is an area where there is much to be learnt.

3.2. MHC class II in pathology

The molecular events that upregulate MHC Class II expression on microglia following disturbance of the local brain homeostasis in pathology are no better understood. In EAE, in which CD4/Th1 T-lymphocytes play a key role, the upregulation of MHC Class II is hardly surprising (Vass et al., 1986). In many of the lesion paradigms described above, however, there are few or no T-cells recruited and thus MHC expression is unlikely to be driven by T-cell secreted γ-interferon. What is of particular interest is that in the rat even stimuli that do not produce neuronal degeneration, for example spreading depression (Gehrmann et al., 1993), result in the upregulation MHC. One possibility is that this is a consequence of activation via K^+ channels (Kettenmann et al., 1990). It should be noted that the contribution of K^+ channels to the regulation of macrophage phenotype is not well studied, and early studies suggest that Cl^- channels rather than K^+ channels may be of relevance (Brown et al., 1998). Studies on microglia activation in the facial nucleus following facial nerve damage, suggest a role for CSF-1 granulocyte–macrophage-CSF (GM-CSF) in the regulation of microglia activation. In the op/op mouse that lacks functional CSF-1 the microglia fail to proliferate following facial nerve transection (Raivich et al., 1994). The microglia rapidly upregulate CSF-1 and GM-CSF receptors during the retrograde response (Raivich et al., 1991). But as noted above, CSF-1 has an immunomodulatory function that suppresses MHC expression

4. APC function in the CNS

4.1. In vitro studies

Induction of EAE in susceptible strains of rodents either by immunisation with myelin proteins or peptides, or by the transfer of autoreactive T-cell lines, requires that the T-cells recognise their antigen within the CNS with the result that they can proliferate and generate the cytokines necessary for disease progression. Histological observations alone cannot provide an answer to which cell is the key player in the presentation of antigen to these T-cells. The first to address this question and a landmark in the study of antigen presentation in the CNS were the experiments by Fontana et al. (1984), in which they demonstrated that astrocytes derived from fetal rats could induce the proliferation of a T-cell line in the presence of soluble MBP. The proliferative response was inhibitable by antibodies to MHC Class II. Since that time, however, the significance of antigen presentation by astrocytes has waned and the role of microglia and perivascular macrophages is clearly in the frame.

Numerous studies have now demonstrated that astrocytes can be induced to express MHC Class II in vitro, although there are important species differences, human derived cells being more inducible than rat (for a review see Sedgewick and Hickey, 1995). These astrocytes, usually derived from fetuses, are able to induce proliferation of T-cell lines, but their capacity to induce primary proliferative responses has not been equally studied and those that have been done suggest that rat astrocytes are unable to support a primary T-cell proliferative response. A recent paper provides evidence that primary murine astrocytes isolated from newborn mice can be induced by γ-IFN to produce a proliferative response not only in primed T-cells but also naive T-cells (Nikcevich et al., 1997). Apart from the obvious possibility of species differences, the authors argue that the mode of isolation, the high levels of B7 co-stimulatory molecules, and finding the appropriate ratio of astrocytes to T-cells are all important factors in primary the antigen presenting capacity of these cells. It should be noted that the naive T-cells were derived from a mouse TCR transgenic mouse, not wild type animals.

If astrocytes can mediate secondary APC functions in vitro, do they express MHC Class II in vivo? After an initial flurry of papers suggesting that astrocytes do express MHC Class II (e.g., Traugott and Raine, 1985; Ransohoff and Estes, 1991), it is now apparent that the levels of expression are either undetectable, or very low. Although these observations apparently leave the status of the astrocytes as a CNS APC unresolved, it appears that astrocytes do not have the capacity to initiate primary proliferative responses, except possibly in mouse and presentation to cells expressing a transgenic T-cell receptor. While it is still possible that astrocytes play a role in secondary responses, a likely and more important role for the astrocyte is the support that these cells provide to microglia and other macrophage populations by their secretion of cytokines during inflammatory states (Eddleston and Mucke, 1993).

In contrast to the status of MHC Class II expression on astrocytes, MHC Class II expression by microglia has been widely and repeatedly demonstrated (see above). It is important to bear in mind the species differences, and the influence of age and systemic infections. The antigen presenting capacity of purified populations of microglia has been demonstrated by several groups (Frei et al., 1987; Matsumoto et al., 1992). The rodent microglia used in these studies were derived from neonatal animals and treated with γ-interferon. The significance of the antigen presenting function of these cells with regard to immunological events in the adult CNS must be questioned. Firstly, it is clear that microglia in the immature nervous system have a very different phenotype from those in the adult, and do not show the highly downregulated phenotype typical of the adult. Secondly, in these studies it was not possible to separate the functional contribution of the parenchymal microglia from the perivascular macrophages, or macrophages associated with the meninges. Given these caveats concerning the isolation of macrophages/micro-

glia from immature brain, the obvious way to proceed is to isolate cells from the adult CNS. Microglia have been isolated from the postmortem human adult nervous system and also from the adult rat brain, but in these studies the phenotype and function of the cells was only examined after culturing in vitro for several days, a time during which the cells can of course de-differentiate (Hayes et al., 1988; Loughlin et al., 1992).

Microglia from adult human brain have also been isolated from tissue derived from surgical resection for the treatment of epilepsy (Williams et al., 1992, 1993). These cells have been reported to act as APCs and mediate not only secondary, but also primary proliferative T-cell responses (Williams et al., 1993). The primary response is somewhat surprising, given results derived from healthy rodent derived cells (see below), and raises the obvious concern as to how the presence of longstanding pathology may have influenced the resident microglia, or indeed the recruitment of other leucocyte populations.

To address these issues, a novel approach has been taken by Sedgwick and colleagues not only to develop a method for the isolation of microglia, separate from other CNS macrophages, but also to use the cells in assays before they have a chance to de-differentiate. Using tissue digestion protocols and flow cytometry, they have shown that the microglia are a distinct $CD45^{low}$ population of cells, whereas the other CNS macrophages from the perivascular, meningeal and choroid plexus compartments are $CD45^{high}$ (Sedgwick et al., 1991; Ford et al., 1995) The $CD45^{low}$ population express low levels of MHC Class II while the $CD45^{high}$ population express higher levels, as is seen immunocytochemically in situ. These two cell populations were then examined for their potential to act as APCs for CD4-positive T-cell lines (Ford et al., 1995). The microglia ($CD45^{low}$) were poor stimulators of T-cell proliferation and IL-2 secretion. The obvious criticism that the method of isolation has interfered with their capacity to present antigen, and generates a proliferative response, is not likely, since the other CNS macrophages ($CD45^{high}$) were as efficient APCs as lymph node APCs isolated as controls. Since the microglia do not express MHC Class II in the normal CNS, this was induced on the microglia in vivo prior to isolation procedures by the induction of systemic graft vs. host disease which is known to upregulate MHC Class II on microglia without a significant cellular infiltrate into the brain (Hickey and Kimura, 1987; Ford et al., 1996). The increased levels of MHC Class II did not significantly affect their ability to induce T-cell proliferation (Ford et al., 1996).

These results show that microglia, contrary to widespread perceptions, are poorly equipped to induce T-cell proliferation. These findings been extended to yet another exciting dimension (Ford et al., 1996). While the microglia do not support the typical signs of T-cell activation, they do induce the T-cells to increase in size, express activation markers IL-2R and OX40, and synthesise the inflammatory cytokines TNF-α and γ-interferon. Despite this the T-cells do not proliferate and in these cultures undergo apoptosis. The apoptosis was found to be preventable by the addition of IL-2, but this did not induce T-cell proliferation, nor were the cells able to be re-stimulated by crosslinking through the T-cell receptor. The microglia are thus presenting antigen in a manner that does not induce T-cell proliferation but actively induces a new state in the T-cell. These findings are in keeping with the observation that many T-cells in the brains of animals with EAE undergo apoptosis within the brain parenchyma (Schmeid et al., 1993), presumably where they are in contact with microglia.

The key message that emerges from these studies is that the microglia may act to protect the tissue from the unwanted attentions of patrolling lymphocytes and rather than encouraging their activation and proliferation actually induce their death. A similar picture to that described by Sedgwick and colleagues for the adult rat microglia has emerged from studies of murine microglia. Microglia isolated from the adult mouse brain express the co-stimulatory molecules B7.2, ICAM and CD40 and can be induced to express MHC Class II (Carson et al., 1998). Despite this repertoire of molecules associated with APCs these microglia are unable to induce proliferation of naive T-cells and the authors conclude that the microglia resemble immature immune cells. Microglia that were derived from mixed glial cultures, as has been described previously, were effective APCs. These studies highlight not only the importance of the CNS microenvironment but the capacity that microglia have to de-differentiate in vitro.

4.2. In vivo studies

The recent in vitro studies described above strongly support the idea that the microglia derived from the parenchyma of the CNS are unable to initiate an immune response to antigen, whether this is a primary or secondary response. In contrast, cells derived from other CNS compartments ($CD45^{high}$) the meningeal and perivascular macrophages are able to do so. We now consider how these in vitro findings relate to the in vivo situation. The in vitro findings emphasise that to study the immune response to an antigen delivered to the CNS, we have to take into account the mode of delivery of the antigen and to which compartments the antigen is, or is not, restricted.

The first studies on transplantation of tissue to the CNS drew attention to the immunological privilege of the brain; tissue transplanted to the brain survived there longer than a comparable graft to a peripheral site. Recent studies have confirmed this partial immunological privilege, but drawn attention to the fact that transplants are rejected, albeit slowly, if immunosuppression is not used (Poltorak and Freed, 1995). The effector arm of the immune system operates in the CNS. It is also clear that transplants to the ventricles are rejected more rapidly than transplants to the

parenchyma (Sloan et al., 1991), however, in neither circumstance has it been defined where the antigen presentation takes place. Do antigens escape from the transplant and sensitisation takes place in peripheral lymphoid organs? Do APCs from the CNS, e.g., perivascular cells or cells with the choroid plexus, carry antigen to the cervical lymph nodes and there initiate a response?

Cserr and colleagues addressed the issue of how soluble antigen delivered to the brain parenchyma might prime the immune system to generate an antibody response. To avoid damage to the blood-brain barrier (BBB) at the time of infusion of the antigen, they first implanted cannulae and allowed the BBB to repair prior to the infusion of xenogeneic albumin or myelin basis protein. They observed that radiolabelled protein rapidly found its way to the deep cervical lymph nodes, although the rate and volume of the material injected was chosen to permit physiological transfer or transport of the protein (Cserr and Knopf, 1992). These studies clearly demonstrated that soluble antigens within the brain parenchyma do drain to lymphoid organs and in the rat, at least, the route involves the cribriform plate. On the basis of these studies, it is easy to conceive that when tissue is grafted to the CNS parenchyma or ventricles, some antigen will find its way to the cervical lymph nodes.

To investigate whether larger particles than soluble protein may find their way to the cervical lymph nodes, xenogeneic erythrocytes have been injected into the parenchyma (Widner et al., 1988). In these studies, however, the volume of material was large and likely to have spilled into several different compartments of the CNS including the ventricle and meninges. The passage of soluble proteins and erythrocytes to the superior cervical lymph nodes is not necessarily representative of the reactions to pathogens, and several studies have investigated the response to the delivery of a pathogen to either the CNS parenchyma, or the ventricles. We (Matyszak and Perry, 1995) chose to use heat killed *Bacillus* Calmette-Guérin (BCG), since there is a large literature on the immune response elicited by this mycobacterium in peripheral organs.

The injection of BCG in a small volume (1 μl) into the ventricles of the rat results in a typical delayed-type hypersensitivity response (DTH) virtually identical to that seen in the skin (Matyszak and Perry, 1996a). In contrast, the injection of the same challenge into the CNS parenchyma provokes a typical acute inflammatory response, but this does not evolve into a DTH response, the BCG remains within the CNS parenchyma for many months (Matyszak and Perry, 1995). The absence of a DTH response is particularly surprising, since the initial injection involves damage to the BBB and elicits recruitment of leucocytes from the blood. Is it possible that the immune system has been sensitised, but the effector arm directed against the BCG is in some way impaired or anomalous? We investigated whether the injection of BCG into the CNS parenchyma sensitises the immune system by looking for evidence of synthesis of antibodies to BCG antigens, the proliferative response to PPD of T-cells isolated from spleen and cervical lymph nodes, and the contact sensitivity to challenge with PPD (Matyszak and Perry, 1997). These experiments showed that the immune system was wholly naive to the BCG within the CNS parenchyma, in marked contrast to the immune system of animals which had received the same challenge subcutaneously. The subcutaneous challenge had sensitised the immune system exactly as one might have expected.

Although BCG sequestered behind the BBB are apparently concealed from the immune system in these experiments, they can provide a target for an immune assault on the CNS. One to five months after the intraparenchymal injection of BCG, some animals were injected with BCG in adjuvant into the hindlimb. There was a prompt and aggressive DTH response at the site of the original BCG deposit within the CNS, resulting in T-cell and macrophage recruitment to the brain parenchyma, breakdown of the blood-brain barrier, primary demyelination and axon damage (Matyszak and Perry, 1995; Matyszak et al., 1997). The lesion bears a striking resemblance to an acute lesion in multiple sclerosis and could be abrogated by anti-inflammatory drugs (Matyszak and Perry, 1996c). This DTH response demonstrates that there are cells within the CNS compartment that were able to present antigen to primed T-cells. Electron microscopic studies using immuno-gold labelling of the BCG, revealed that the BCG was present within both perivascular macrophages and microglia within the parenchyma (Matyszak and Perry, 1997). An interesting feature of these lesions was the recruitment of large numbers of DCs into the brain parenchyma as identified by immunocytochemistry for the OX62 antigen and electron microscopy (Matyszak and Perry, 1996b). Whether these DCs are competent to ingest proteins within the microenvironment of the CNS and migrate back to the cervical lymph nodes is not known.

This series of in vivo experiments demonstrates that there are no cells in the CNS parenchyma that can elicit a primary immune response, and indeed it appears that the perivascular cells are also unable to elicit a primary response. Either the microglia, or more likely the perivascular macrophages, are able to support a secondary response. These results are wholly consistent with the in vitro studies described above. It might be argued that our failure to find a response is due to the absence of 'sufficient levels' of MHC Class II on cells within the parenchyma. We have investigated whether upregulation of MHC has any influence on the response to parenchymal injection of BCG, by injecting γ-interferon prior to the BCG injection (Matyszak and Perry, unpublished observations). As earlier, there was no DTH response to this injection.

It has been suggested that BCG is an unusual antigen, and is itself an adjuvant: this makes the findings all the more surprising. However, other experiments have demon-

strated the same absence of a primary response to a pathogen delivered and restricted to the brain parenchyma. It has been previously shown that intracerebral inoculation with virus can lead to rapid dissemination of the virus into the CSF and the blood stream (Mims, 1959). These studies provide little control over sites of immune activation, and thus little insight into APC function in the CNS. Stevenson et al. (1997) have, however, investigated how the immunogenicity of influenza virus depends on the site of inoculation. Large volume injections (5–30 μl) into the CNS parenchyma or ventricles, give a comparable immune response, antiviral T-cell proliferative response and antiviral antibody synthesis, to that seen with intranasal challenge. However, when a small volume of virus (0.5 μl) was delivered and restricted to the CNS parenchyma, there was no detectable immune response over the first 10 days, and 54% of the mice had no detectable response up to 80 days later. These studies once again show the importance of taking into account the different compartments of the CNS when considering the initiation of the immune response.

5. Conclusion

The CNS has a number of discreet compartments within which the microenvironment has a potent influence on the resident phagocyte populations and their capacity to effectively initiate or support an immune response. While the proliferation of T-cells has commonly been used as evidence of antigen presentation, it is clear that antigen presentation may lead to cytokine production without proliferation, or even T-cell death. In vivo studies show that delivery of a foreign antigen to the meninges or ventricles results in a robust immune response indicating that antigen may readily drain from this site to lymphoid organs, or that there are cells present capable of initiating a primary immune response. In contrast, the delivery of a pathogen restricted to the parenchyma of the brain does not give rise to an immune response, thus indicating that there is insufficient drainage to regional lymph nodes and that there are no cells capable of initiating a primary proliferative response. There is, however, a population of cells associated with the vasculature of the brain parenchyma, the perivascular macrophage, that is a likely candidate for the initiation of secondary T-cell proliferative responses. In vitro studies provide evidence that microglia, although unable to present antigen in a context that initiates a proliferative response are able to present antigen in a manner that induces T-cell cytokine synthesis or T-cell death. The microglia are thus able to play a key role in terminating T-cell responses against self antigens. The protective role of the microglia has a cost associated with it, namely that pathogens may remain sequestered behind the blood-brain barrier hidden from the immune system. Peripheral sensitisation to the same pathogen may, however, lead to a reaction against the sequestered pathogen. These in vitro and in vivo observations have implications for our understanding, not only of immune mediated diseases of the CNS, but also gene therapy and tissue transplantation within the CNS.

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