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Persistent pathogens in the parenchyma of the brain

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It has recently been shown that bacteria and viruses can be delivered to the brain parenchyma without evoking an immune response. These experiments demonstrate that there are no cells within the brain parenchyma that can initiate a primary immune response, and that the drainage of pathogens from the brain parenchyma is distinct from that documented for soluble proteins. A persistent pathogen in the brain parenchyma can become a target for the immune system following peripheral sensitisation, and this may lead to bystander tissue damage. These observations may have consequences for vaccination of persons with central nervous system HIV infection. *Journal of NeuroVirology* (2000) **6,** S86–S89.

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Microglia are the resident macrophages of the central nervous system. When compared to other tissue macrophages the microglia have a switched off or downregulated phenotype (Perry, 1994). In addition to the microglia there are other populations of macrophages associated with the CNS namely the perivascular cells (Graeber *et al*, 1989) and the macrophages of the choroid plexus and meninges (Matyszak *et al*, 1992). The physiological relevance of the downregulated phenotype of the microglia is not well understood but the dementia associated with HIV clearly highlights the importance of this phenotype. In HIV-dementia, the infected microglia and possibly also neighbouring uninfected cells, become activated and these activated microglia secrete a spectrum of molecules that may cause neuronal dysfunction or death (Gendelman *et al*, 1994). Microglia become activated following diverse insults and injuries to the brain and in both rodent and human CNS upregulate, or synthesise *de novo,* antigens that are not expressed by microglia in the normal brain. The appearance of MHC Class II on microglia has repeatedly led to the suggestion that microglia are the antigen presenting cells (APCs) of the CNS. In contrast, there is evidence that it is the perivascular macrophages that are the APCs of the CNS (Hickey and Kimura, 1988). Although it is possible to isolate the microglia from the CNS and investigate their antigen presenting capacity *in vitro*, unless this is done rapidly, and under the appropriate conditions, the cells de-differentiate *in vitro* (see below). We have thus addressed the question *in vivo*. Are there cells in the CNS parenchyma that are able to initiate a primary immune response?

To explore this question we injected bacillus Calmette-Guérin (BCG) directly into the CNS. By using focal injections of the mycobacterium, in a volume of one microlitre or less, we can study whether there are differences in the responsiveness of the different compartments of the brain, for example, the ventricles *versus* the parenchyma. The differential innate inflammatory response in the ventricles, meninges, and parenchyma following challenge with pro-inflammatory agents has been well demonstrated (Anderson *et al*, 1992; Anthony *et al*, 1997). The injection of endotoxin, or interleukin-1β, into the adult brain parenchyma produces a florid inflammatory response in the meninges but only a very modest inflammatory response in the parenchyma.

Injection of heat-killed BCG into the ventricles of the rat produces an acute inflammatory response that evolves into a delayed-type hypersensitivity (DTH) response over a period of several days (Matyszak and Perry, 1996a). This response is essentially indistinguishable from that seen in the skin. When heat-killed BCG are injected into the brain parenchyma (carefully avoiding the ventricular system) there is an acute inflammatory response, as we might expect, but in marked contrast to the ventricles or skin there is no subsequent T-cell recruitment and the inflammatory response does not develop into a typical DTH response: the blood-brain barrier repairs and the recruited myelomoncytic cells are cleared (Matys-

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zak and Perry, 1995). Immunocytochemical studies demonstrate that the BCG is phagocytosed by cells of the mononuclear phagocyte lineage and it may remain within these cells for up to 1 year after the injection (Figure 1a). There is no evidence of microglia activation in the region of the BCG deposit (Figure 1b). Ultrastructual studies and immunogold labelling demonstrate that the BCG remains within phagolysosomes of both perivascular macrophages and microglia (Matyszak *et al*, 1997).

The absence of a typical DTH response within the brain parenchyma shows that there are no cells in the brain parenchyma that are able to carry antigen back to the lymphoid tissue and present it naïve T-cells. There are, thus, no dendritic cells (Steinman, 1991) in the brain parenchyma that can initiate a primary immune response although there are such cells in the choroid plexus and meninges (Matyszak and Perry, 1996b; McMenamin, 1999).

The absence of a DTH response against the BCG deposited within the brain parenchyma is somewhat surprising given that soluble antigens such as albumin have been shown to rapidly drain from the brain parenchyma to the cervical lymph nodes (Cserr and Knopf, 1992). We therefore looked for evidence that the BCG within the brain might have induced an atypical immune response that was not expressed as a typical DTH response (Matyszak and Perry, 1998). Injection of BCG into the pinna of the ear leads to synthesis of antibodies to PPD, which are readily detected in the serum. Following the same BCG challenge to the pinna, T-cells isolated from spleen and cervical lymph nodes proliferate when confronted with PPD, and a contact sensitivity response to PPD is readily demonstrated in the ear opposite to the BCG deposit. However, injection of the same amount of BCG into the brain parenchyma failed to sensitise the immune system and no antibodies to PPD were generated, there was no evidence from T-cell proliferation assays or contact sensitivity assays that the immune system had seen BCG antigens (Matyszak and Perry, 1998). In essence the immune system was wholly naive to the presence of the BCG within the brain parenchyma. The absence of an immune response to the BCG within the brain parenchyma would appear to be at variance with studies on antigen drainage from the brain (Cserr and Knopf, 1992) but of course bacteria, and viruses, are not soluble proteins. The lack of responsiveness in the CNS parenchyma is not restricted to heat-killed BCG but has also been found after small injections of heat- killed Cornyebacterium parvum and live BCG (Mastyzak and Perry, unpublished observations), a replication-deficient adenovirus (Brynes *et al*, 1996) and influenza virus (Stevenson *et al*, 1997). The differential immune response to pathogens in the CNS compartments has obvious parallels with the immune response to tissue transplantation in the CNS (Sloan *et al*, 1991).

The isolation from the immune system is not absolute. In animals with a BCG deposit within the brain parenchyma a subsequent peripheral challenge with BCG will lead to a DTH response at the site of the initial BCG deposit within the brain, (Matyszak and Perry, 1995). T-cells and macro-

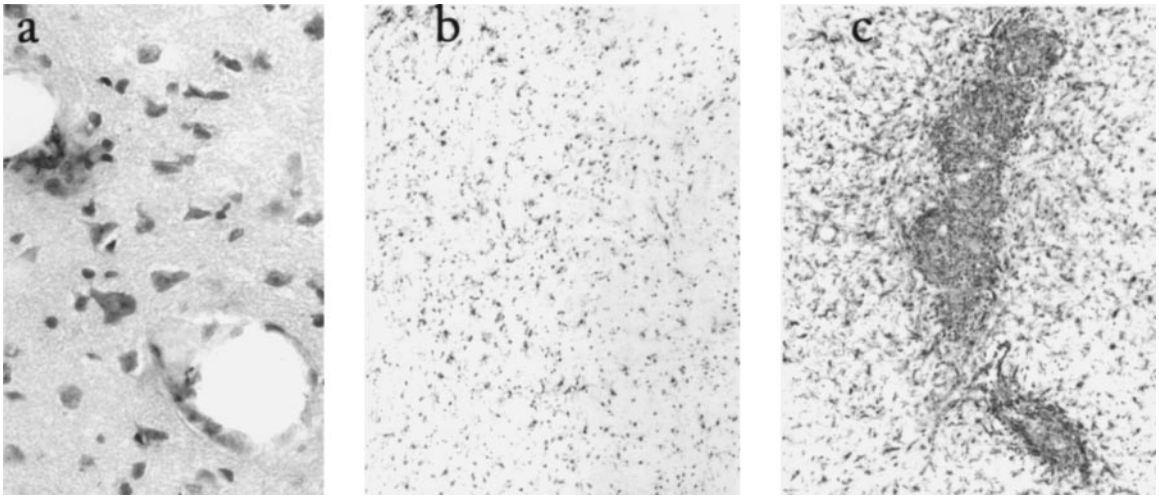

Figure 1 Photomicrographs to illustrate how heat-killed BCG may remain sequestered behind the blood-brain barrier without evoking a DTH response, but may act as a target for a DTH following peripheral challenge. (**a**) Immunocytochemical localisation of BCG in perivascular macrophages 1 year after injection into the brain parenchyma. Note the absence of any leucocyte cuffing around the vessels that would be indicative of a DTH response. (**b**) The resident microglia in the region of the BCG deposit shown in (**a**) are revealed with antibodies against the complement receptor type 3 (OX42). The microglia show no evidence of activation. (**c**) One year after the injection of heat-killed BCG into the brain parenchyma the animal was injected subcutaneously with BCG to sensitise the immune system. Two weeks later a typical DTH response is present at the site of the original BCG deposit in the brain with large numbers of macrophages and T-cells forming a lesion.

phages infiltrate the tissue and around the granuloma there are many activated microglia (Figure 1c). The recruited leucocytes damage the blood-brain barrier and damage the neural tissue, both myelin and axons. It is clear that there are cells within the brain parenchyma that are able to present the BCG antigens to the peripherally primed T-cells as we might expect from numerous studies an experimental allergic encephalomyelitis (Wekerle, 1993). In peripheral tissues there are a number of cell types that can support a secondary immune response and several cell types have been suggested to act as antigen presenting cells in the brain parenchyma. Astrocytes have had a somewhat chequered history in regard to their role as APCs but the absence of MHC Class II antigen on these cells *in vivo* makes it unlikely that they are directly involved in presentation to CD4 T-cells (see Sedgwick and Hickey, 1996 for review). Although it is commonly stated that microglia are the antigen presenting cells in the brain it seems unlikely that they are responsible for T-cell proliferation in these secondary responses.

Sedgwick and colleagues have developed a protocol that allows the microglia to be rapidly isolated from the brain so as to maintain their phenotype (Sedgwick *et al*, 1991; Ford *et al*, 1995). The microglia are $CD45^{low}$ expressing cells in contrast to the $CD45^{high}$ population which are likely to be perivascular macrophages and some residual meningeal macrophages. The $CD45^{high}$ cells will induce proliferation of a myelin basic protein T-cell line in the presence of antigen. The $CD45^{low}$ cells do not induce T-cell proliferation but instead induce a state of anergy and even direct a percentage of the T-cells to undergo apoptosis (Ford *et al*, 1996). Similar results demonstrating the lack of T-cell proliferation have been obtained with microglia isolated from the mouse brain (Carson *et al*, 1998). Thus, the notion that microglia are the APCs of the CNS that induce T-cell proliferation is clearly not correct and these experiments show rather that the microglia may play an important role in protecting the brain parenchyma from the unwanted attentions of autoreactive T-cells that enter the tissue.

In the normal brain there are no dendritic cells within the brain parenchyma. However, following the initiation of a DTH response in the brain parenchyma directed against the BCG and in animals with EAE there are significant numbers of OX62 positive cells within the lesions (Matyshak and Perry, 1996b). The antigen recognised by OX62 is an integrin present on dendritic cells (Brennan and Puklavec, 1992). The factors that recruit the dendritic cells to these lesions have not been studied. It remains an intriguing question as to whether these dendritic cells can traffic back to lyphoid tissue bearing CNS antigens and present these antigens to naïve T-cells.

Conclusion

These experiments have shown that there are no antigen presenting dendritic cells within the CNS parenchyma although cells with this capacity exist within the ventricular and meningeal compartments. Thus, a pathogen may remain in the brain parenchyma undetected by the immune system. The entry of a pathogen into the brain parenchyma without prior recognition by the immune system could occur in sub-clinical infections or in immunocompromised individuals. Vaccination against the pathogen, or recovery of a compromised immune system, with subsequent recognition of the antigen peripherally may lead to an immune assault on the pathogen in the brain with bystander tissue damage. These observations may be relevant to the HIV seropositive individual who may not only harbour HIV within the brain but also a number of other pathogens.

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