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# Infiltration of immune T cells in the brain of mice with herpes simplex virus-induced encephalitis

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## Summary

Herpes simplex virus (HSV) infection of mice can induce viral encephalitis. Using two-fluorochrome immunofluorescence, our present study shows that though there is extensive myelin loss and necrosis in the brain stem of mice with HSV encephalitis, only some oligodendrocytes, astrocytes and microglial cells are infected. T cells that express CD4 or CD8 and a large number of $CD4^+$, $F4/80^+$ macrophages are present in perivascular infiltrates close to and in contact with HSV-infected cells in areas of massive myelin loss. These findings suggest that the resultant infiltration of immune cells into the brain during HSV-1 infection may cause as much damage as the virus itself.

## Introduction

Herpes simplex virus (HSV) is a neurotropic virus. Severe infection of man and mice with the virus can result in acute encephalitis and mortality. Depending on the route of infection, the pathology of the disease is situated in the peripheral or central nervous systems.

While the protective effect of T cell responses against HSV-1 infection has been extensively studied, especially with respect to the role of $CD4^+$ (Chan et al., 1985; Nash et al., 1987) and $CD8^+$ cells (Nash et al., 1980; Nagafuchi et al., 1982; Larsen et al., 1983; Nash and Gell, 1983; Sethi et al., 1983), less is known of the effect of T lymphocytes on the pathology of HSV-1-induced disease. It was demonstrated that after corneal infection of HSV-1, athymic mice suffered milder CNS pathology than do immunocompetent mice (Townsend, 1981), suggesting that T cells may produce significant central nervous system (CNS) damage. Similarly, immunosuppression of mice with cyclophosphamide prior to infection resulted in a marked reduction of mononuclear infiltrates and myelin destruction in the CNS compared with non-immunosuppressed mice, though both groups of mice showed comparable virus and serum neutralising antibody titres (Townsend and Baringer, 1979; Altmann and Blyth, 1985). These studies

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suggest that the immune response triggered during HSV-1 infection and the resultant infiltration of immune cells into the CNS can, under certain circumstances, cause as much damage as the virus itself. While previous reports have shown cellular infiltrates into the brain during HSV-1 infection (Townsend and Baringer, 1979; Townsend, 1985), the cell types have not been identified or characterised.

The questions we have addressed here are whether myelin loss is related to the type and proportion of neural cells infected with HSV, what cells infiltrate into the brain during infection and how do they relate spatially to infected cells or areas of myelin loss. In this study, therefore, we have used cell type-specific markers which would allow unambiguous identification of the different types of neural or immune cells in the brain. Glial fibrillary acidic protein (GFAP) is an astrocyte-specific protein (Bignami et al., 1972), galactocerebroside (GC) is the major galactosphingolipid of myelin on the surface of oligodendrocytes while CD8 and CD4 are specific cell surface molecules of cytotoxic/suppressor and helper (inducer) T cells respectively. The rat monoclonal antibody F4/80 is directed against a 160000 Da plasma membrane glycoprotein on mature mouse macrophages (Austyn and Gordon, 1981). By marking specific cell types with antibodies conjugated to one fluorochrome, we have been able to study the expression of HSV or CD4 antigen on these cells or of their spatial relationship to other HSV-infected cells by using antibodies conjugated to a different fluorochrome. We report here evidence obtained from double-label indirect immunofluorescence that oligodendrocytes, astrocytes and microglial cells in the brain stem of mice showing clinical signs of viral encephalitis are infected with HSV-1. A large number of $CD4^+$, $F4/80^+$ macrophages and some T cells that express CD4 or CD8 are present in perivascular infiltrates in the vicinity as well as in contact with HSV-infected cells and this is also an area of massive myelin loss.

## Materials and methods

Male CBA mice, 7–8 weeks old and obtained from OLAC, U.K., were infected subcutaneously (s.c.) in the right ear with $6 \times 10^6$ plague forming units (pfu) of HSV-1 Kruegger. They were sacrificed when they showed clinical signs of viral encephalitis. The brain was immediately removed, embedded in O.C.T. compound (Miles Scientific, U.S.A.) and horizontal cryostat sections 15 $\mu$m thick were made. For histological examination, sections were fixed in calcium formalin and absolute alcohol before being stained with solochrome cyanin, washed with 4% ammonium ferric sulphate and counter-stained with eosin. For immunofluorescence examination, sections were fixed in cold acetone ($-20\,^\circ$C) for 10 min.

All the antibodies used in this study have been previously described or were purchased. The mouse monoclonal anti-GC antibody (Ranscht et al., 1982) was a gift from Dr. M.C. Raff and was used as ascites fluid diluted 1:50. The respective anti-CD8 and anti-CD4 (anti-L3T4) rat monoclonal antibodies, YTS 169.4 (Cobbold et al., 1984) and GK 1.5 (Dialynas et al., 1983) were a gift from Dr. F.Y. Liew and used as ammonium sulphate precipitate diluted 1:10. The F4/80 rat monoclonal antibody specific for mature mouse macrophages (Austyn and Gordon, 1981) was a gift from Dr. S. Gordon via Dr. P. Kaye and was used as hybridoma culture supernatant diluted 1:1. The rabbit anti-HSV and anti-GFAP sera were both obtained from Dako and used at 1:100 and 1:200 respectively. The binding of the mouse monoclonal anti-GC was detected by indirect immunofluorescence using rhodamine-conjugated goat anti-mouse immunoglobulin (G anti-MIg-Rd, Cappel, diluted 1:50); rat monoclonal antibodies were detected using fluorescein isothiocyanate (FITC)-conjugated goat anti-rat immunoglobulin (G anti-RatIg-FITC, Cappel, diluted 1:100) for YTS 169.4 and GK 1.5; and biotin-conjugated goat anti-rat immunoglobulin and avidin-conjugated rhodamine (biotin-G anti-RatIg and Av-Rh, Sigma, diluted 1:50 and 1:100 respectively) for F4/80. The binding of the polyclonal rabbit anti-HSV and rabbit anti-GFAP antibodies was detected with biotinylated donkey anti-rabbit immunoglobulin and avidin-coupled Texas red (D anti-RIg-biotin and Av-Tx red, Amersham, diluted 1:50 and 1:100 respectively) and mouse anti-HSV immunoglobulin with G anti-MIg-Rd, Cappel, diluted at 1:50.

Cryostat sections were double-labelled as described by Raff et al. (1979). Sections were exposed to the primary and secondary antibodies sequentially in the presence of 0.1% Triton X-100 and 10% normal serum. Three 10 min washes were performed between each incubation. All incubations were for 1 h at room temperature. After the last wash, sections were mounted in glycerol and sealed with nail varnish before they were examined in a Zeiss Universal incidence fluorescence microscope equipped with phase-contrast, fluorescein, and rhodamine optics and photographed using Kodak TMX 5052 or Ektachrome colour-slide film rates 400 ASA.

## Results and discussion

There was an extensive loss of myelin in the brain stem of mice during acute viral encephalitis (Fig. 1*b* and *c*) compared with no loss in the normal brain (Fig. 1*a*). Foci of myelin loss (fine arrow) were seen to be associated with and in close proximity to large perivascular infiltrates (thick arrow). No cellular infiltrates were evident in the normal brain. This suggests that such infiltrates may by an immune attack contribute to the widespread and extensive myelin destruction in this area apart from the direct viral cytopathic effect on relevant glial cells like oligodendrocytes.

In order to determine which cells are infected, sections were double-labelled with anti-HSV antibody and with antibodies specific for the glial surface markers, galactocerebroside of oligodendrocytes and GFAP of astrocytes. Some oligodendrocytes (Fig. 2) and astrocytes (data not shown) were infected. Since oligodendrocytes are myelin-forming cells while astrocytes reportedly function as physical and nutritional supporting cells for other glial cells, it is conceivable that infection of such cells and the resultant cytocidal effect would contribute to myelin loss. However, although there was extensive myelin destruction accompanied by necrosis within the brain stem, the proportion of infected glial cells was small. It is therefore unlikely that myelin loss is due entirely to infection of glial cells, and further studies were made of the infiltrating cells which were concentrated in the infected areas.

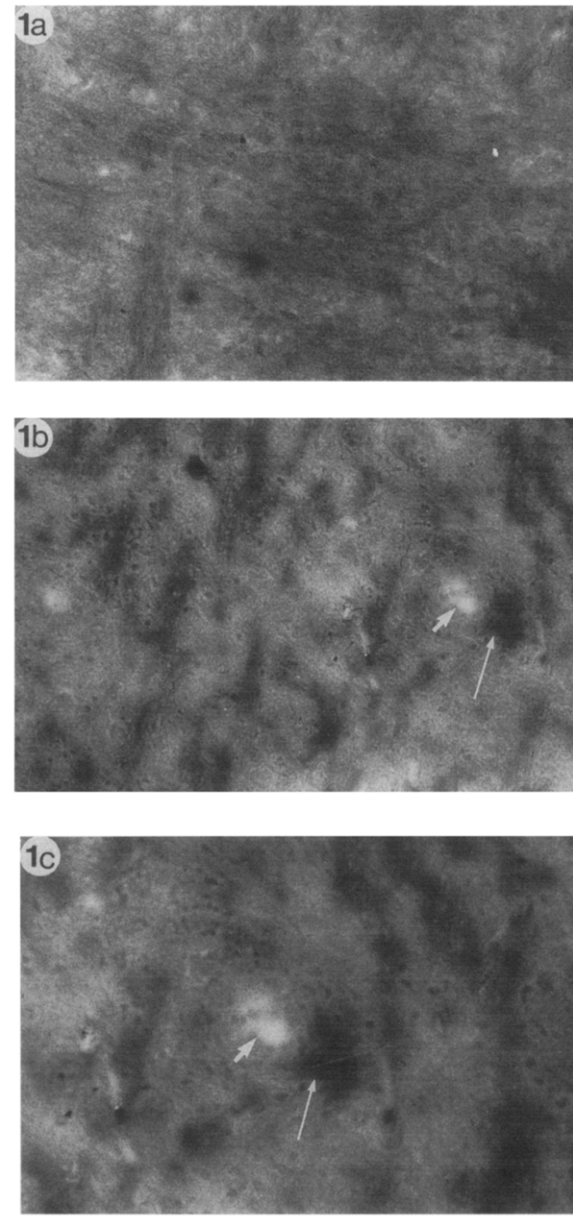


Fig. 1. Horizontal cryosection of mouse brain stem stained for myelin with solochrome-cyanin. (*a*) Normal mouse brain and (*b*) brain of mouse with viral encephalitis. Note the foci of myelin loss (fine arrow) associated with the perivascular infiltrates (thick arrow). (*c*) High power of (*b*) indicating perivascular infiltrate of rounded cells. Counterstained with eosin, magnification ×16 and ×25.

Monoclonal antibodies YTS 169.4 (anti-CD8), GK 1.5 (anti-CD4) and F4/80 (a mature mouse macrophage marker) were applied in a double-label

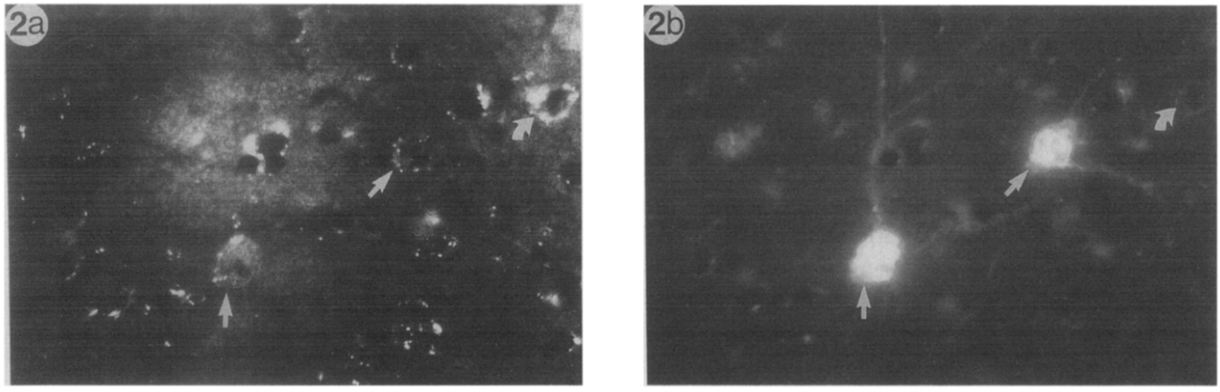


Fig. 2. Infected oligodendrocytes in brain stem of mouse with viral encephalitis. Immunofluorescent double labelling with anti-GC (*a*) and anti-HSV (*b*) antibodies. The $GC^+$ cells which are strongly $HSV^+$ (straight arrow) show sparce speckled fluorescence with the anti-GC antibody, suggestive of myelin loss, whereas the uninfected $GC^+$ cell (curved arrow) has a much more intense and uniform fluorescent pattern. Note the distribution of HSV antigen on the cell body and processes of the infected oligodendrocytes.

technique to localize cytoxic/suppressor T cells, helper (inducer) T cells, and macrophage/monocytes respectively relative to HSV-infected cells in the sections. As shown in Fig. 3, $CD8^+$ cells (straight arrow) occur perivascularly in small clusters in the vicinity of necrotic areas of infected cells (curved arrow) and they also occur individually or in groups of a few cells in the brain parenchyma. Occasionally they can be seen next to an infected cell. In contrast, the $CD4^+$ cells (broad arrow in Fig. 4) occur in large numbers as rounded lymphocyte-like cells localised mainly in perivascular cuffs. Again these are mainly in the vicinity of necrotic areas of infected cells in the brain stem and occasionally a $CD4^+$ cell (arrowhead) can be seen in contact with an infected cell (Fig. 4*a* and *b*). Though the majority of $CD4^+$ cells are not infected, some of them (curved arrow) appear to be (Fig. 4*a* and *b*). This is in agreement with recent evidence that T lymphocytes when activated can support otherwise non-permissive HSV replication (Braun and Kirchner, 1986). Like the $CD4^+$ cells, the $F4/80^+$ cells are localised as rounded cells in perivascular infiltrates in the vicinity of infected cells and some of them are infected (data not shown). In addition, there is also a diffuse distribution of $F4/80^+$ microglial-like cells in the parenchyma (Fig. 5*a*). Such distribution and morphology is in sharp contrast to the less abundant $F4/80^+$ mi-

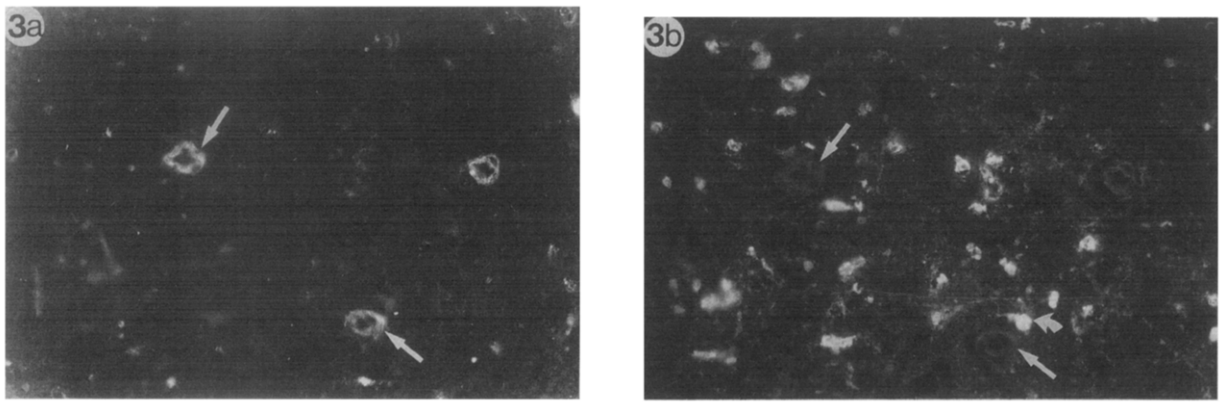


Fig. 3. Perivascular infiltrates of $CD8^+$ cells (straight arrow) in the vicinity of HSV-infected cells (curved arrow) in the mouse brain stem. Immunofluorescent double labelling with anti-CD8 (*a*) and anti-HSV (*b*) antibodies. Note that the $CD8^+$ cells are HSV negative despite their close proximity to HSV-infected cells.

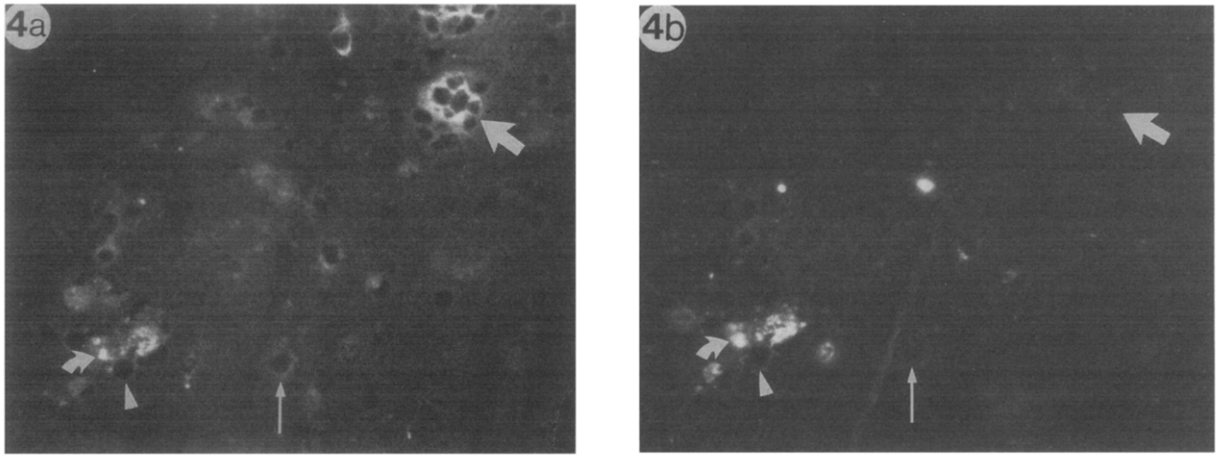


Fig. 4. $CD4^+$ cells (broad arrow) as clusters of perivascular infiltrates in the vicinity of HSV-infected cells in the mouse brain stem. Immunofluorescent double labelling with anti-CD4 (*a*) and anti-HSV (*b*) antibodies. Note that some $CD4^+$ cells (fine arrow) are also present as single cells in the brain parenchyma. Some of the infected cells are $CD4^+$ (curved arrow).

croglial cells present in the brain stem parenchyma of normal mice (data not shown). The similar distribution seen with the $CD4^+$ and $F4/80^+$ cells in the infected sections and the large number of $CD4^+$ cells detected prompted us to investigate this further. Using double labelling, it was seen that among the perivascular infiltrates, all the rounded $F4/80^+$ cells (arrow) were also CD4 positive (Fig. 5*a* and *b*) whereas only some of the $CD4^+$ cells were F4/80 negative (data not shown). Most other $F4/80^+$ microglial-like cells in the parenchyma were CD4 negative (Fig. 5*a* and *b*). This result is consistent with a recent report (Perry and Gordon, 1987) that monocytes and macrophages express CD4 antigen, and that microglial cells can modulate CD4 antigen levels after inflammation and changes in the blood–brain barrier. Such CD4 antigen expression appears to coincide with a change in morphology of microglia and may represent a process of cellular activation. Because of this, it is conceivable that some of the $CD4^+$-infected cells seen may be $CD4^+$ microglial cells. Therefore HSV infection of brain cells can lead to an accumulation of macrophages which may be due to an invasion and proliferation of blood-derived monocytes and/or activation of microglial cells.

Our studies show that the massive cellular

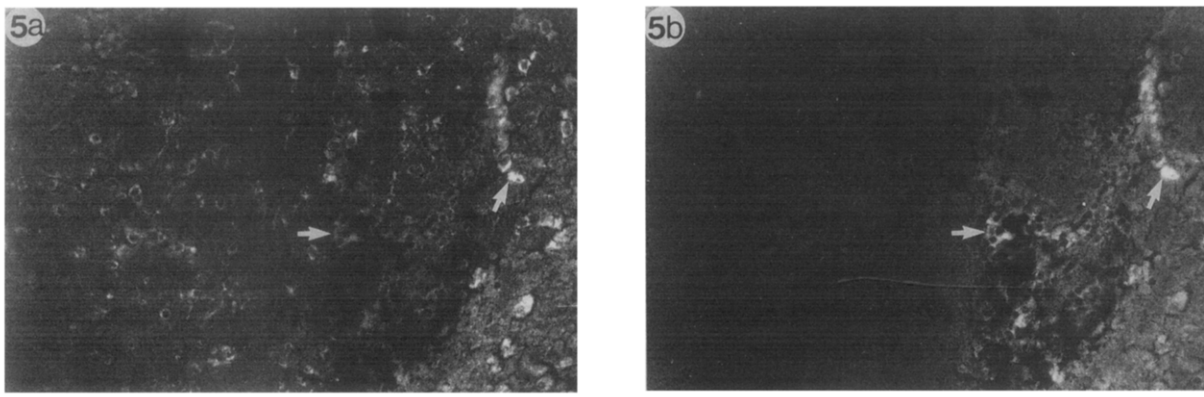


Fig. 5. Distribution of $F4/80^+$ cells that are $CD4^+$ in the brain stem of infected mice. Immunofluorescent double labelling with anti-F4/80 (*a*) and anti-CD4 (*b*) antibodies. Note that most $F4/80^+$ microglial-like cells in the brain parenchyma are $CD4^-$ while all the rounded $F4/80^+$ cells in perivascular areas are $CD4^+$ (arrow).

infiltrates at the site of lesions and myelin destruction contain $CD4^+$, $CD8^+$ and $F4/80^+$ cells. These findings suggest that immune cells may be involved in myelin destruction and are consistent with previous reports that T cells may produce significant CNS pathology during HSV encephalitis (Townsend and Baringer, 1979; Townsend, 1981; Altmann and Blyth, 1985). The precise role of these cells in the pathology of HSV-1-induced encephalitis is at present unclear. It is, however, likely that this may be mediated via lymphokines.

Activated macrophage damage to myelin may be by direct contact or by the production of neutral proteases which can degrade myelin basic protein in vitro (Cammer et al., 1978). Since encephalitogenic T cell clones specific for myelin basic protein have been shown to induce experimental autoimmune encephalomyelitis (EAE) and glial cells can be induced to express MHC class I or class II antigen (Ting et al., 1981; Hirsch et al., 1983; Fontana et al., 1984; Wong et al., 1984; Fierz et al., 1985; Massa et al., 1986; Susumura et al., 1986), it is conceivable that such cells may present viral antigen or myelin basic protein as a bystander effect to class II-restricted $CD4^+$ encephalitogenic T cells (Yasukawa and Zarling, 1984; Sun and Wekerle, 1986) or to class I-restricted $CD8^+$ cytotoxic cells, both of which type of T cell subsets are present at the lesion.

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