Brief Definitive Report

MODULATION OF CD4 ANTIGEN ON MACROPHAGES AND MICROGLIA IN RAT BRAIN

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During development circulating monocytes are recruited to the central nervous system (CNS) to dispose of degenerating neurons, after which monocyte-derived cells persist throughout adult life as microglia and other specialized macrophage (MØ) populations, e.g., in the choroid plexus (1). After injury or inflammation there is increased recruitment from blood and a poorly defined local change in the morphology of microglia known as reactivation (2). The MØ-specific mAb F4/80 labels all forms of MØ in the CNS, whereas monocyte antigen (Ag) such as the leukocyte common (LC) Ag are poorly expressed by microglia, suggestive of local modulation during maturation.

Recently, it has been proposed that monocytes and MØ play an important role in the widespread neurologic complications that follow HIV (AIDS) infection in man. The virus in the CNS has been detected mainly within MØ giant cells and monocytes. This finding is consistent with evidence that monocytes and MØ express authentic CD4 Ag (3, 4), which serves as a receptor for HIV entry into cells of the immune system (5). However, there is still considerable uncertainty as to which cells in the CNS express CD4 Ag and it is not known how CD4 levels vary in the normal and infected host. Since rat monocytes and MØ display surface CD4 Ag (3), we have studied expression of this and other leukocyte Ag in the developing and adult rat brain and after injury to learn more about modulation of MØ CD4 Ag in a defined experimental model. We report here that CD4 Ag in normal rat brain is restricted to MØ and microglia, that Ag expression is modulated by microglial differentiation and reactivation, and that the blood brain barrier may contribute to selective downregulation of CD4 on mature microglia.

Materials and Methods

Immature (1, 3, 5, 15 d) and adult Long Evans rats were studied untreated or 3–10 d after a local cortical lesion, ~1 mm in diameter, made by aspiration in the dorsal cortex under chlornembutal anesthesia. Animals were perfused through the heart with periodate-lysine-paraformaldehyde, 10-μm cryostat sections were cut in the coronal plane, and sections at different levels between the optic chiasm and the superior colliculus were saved. These were incubated with various mAbs and binding was detected by the avidin-biotin-complex immunoperoxidase method (1). Mouse mAbs used included W3/25 and

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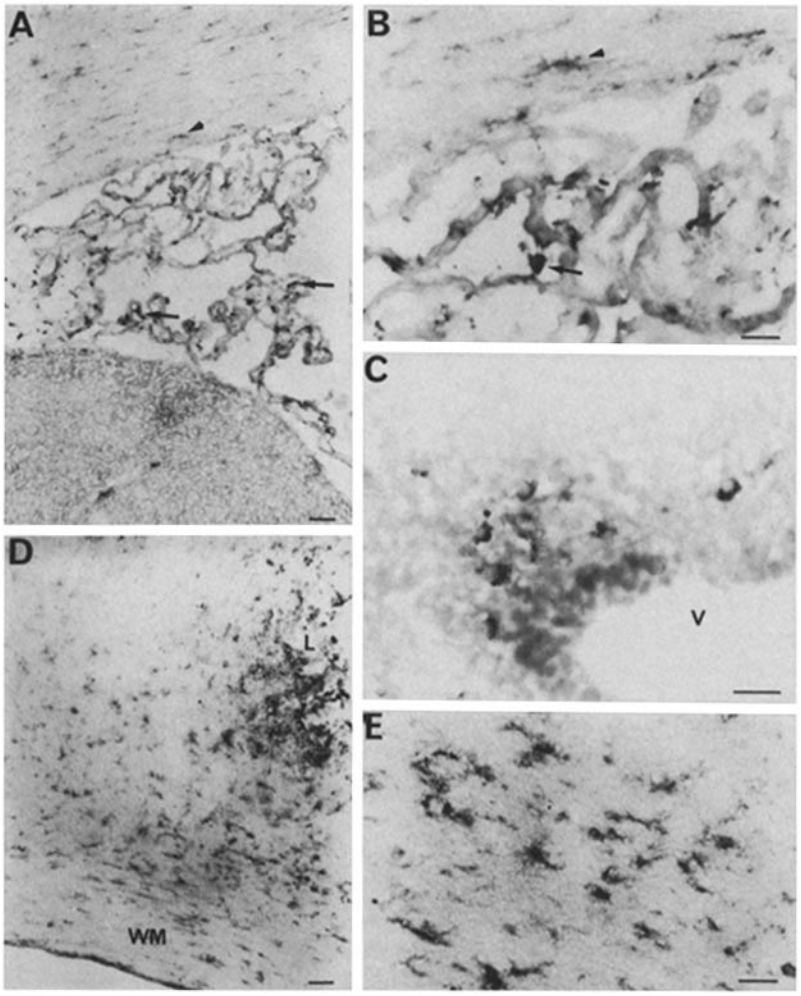

FIGURE 1. Photomicrographs of W3/25$^+$ cells in rat brain. (*A*) Normal adult rat choroid plexus. Note the many W3/25$^+$ cells (*arrows*) within the plexus and a few cells on the ventricular surface. Some microglia in the adjacent white matter (*arrowhead*) are also W3/25$^+$. (*B*) High power of *A* indicating rounded W3/25$^+$ cell in the ventricle (*arrow*) and microglial cells (*arrowhead*) in the white matter. (*C*) W3/25$^+$ cells adjacent to ventricle (*V*) in a 5-d-old rat. (*D*) W3/25$^+$ cells in cortex 5 d after a small aspiration lesion (*L*). Numerous W3/25$^+$ monocytes are found within the lesion site. Microglia in the grey matter adjacent to lesion and in the white matter (*WM*) are now intensely stained, compare with *A*. (*E*) High power of *D* showing intensely stained microglia adjacent to the lesion. Note the extensive processes. Scale bars in *A* and *D* = 50 μm; *B*, *C*, and *E* = 25 μm.

OX35, directed against different CD4 Ag epitopes (3), OX1 and OX30, against different LC Ag epitopes (7), OX42 (iC3b receptor) (6), OX8 (CD8), OX19 (found on T lymphocytes and thymocytes), and OX26 (7) (transferrin receptor). These mAb were either IgG1 or IgG2a isotypes and appropriate class controls excluded binding to MØ Fc receptors, which are destroyed by this fixation protocol.

Results and Discussion

Fig. 1 illustrates results obtained with W3/25. In the normal adult, this mAb consistently stained rounded MØ in the choroid plexus, occasionally within the

ventricle (Fig. 1, *A* and *B*), and in the leptomeninges (not shown). These were identical in morphology and distribution to cells detected in rat brain by an MØ-specific polyclonal rabbit F4/80 antiserum, which in addition marked developing and mature microglia, as in mouse brain (not shown). Only a small number of rat microglia were W3/25$^+$, as illustrated in the same Figure (Fig. 1, *A* and *B*), in all regions examined. Microglia in the white matter were weakly but consistently W3/25$^+$ while those in the grey matter were infrequently W3/25$^+$. A similar picture was obtained with OX35, another CD4 marker, used alone or together with W3/25. LC staining was close to the limits of detection on most microglia. An occasional isolated rounded W3/25$^+$, LC$^+$ cell was found outside blood vessels throughout grey and white matter and some of these had the morphology of lymphocytes. The T lymphocyte markers OX8 and OX19 were not detected on monocytes, MØ, or microglia. A few rounded cells, presumed to be T lymphocytes, were found in choroid plexus, meninges, and in the ventricles but very rarely in brain parenchyma. OX26 has been used previously to stain brain capillaries selectively (7). We confirmed that this mAb could stain capillary endothelium in brain and also noted very weak labeling of some microglia. However, choroid plexus capillaries were OX26$^-$, and the OX26$^+$ brain capillary endothelium was unmarked by W3/25.

These studies established that in normal adult rats, CD4 Ag was restricted to monocytes, MØ, and occasional lymphocytes, and was present but weak on microglia. These conclusions were confirmed by examination of newborn animals. As found previously in the mouse (1), groups of rounded MØ were found widely scattered in cortical white matter and were especially prominent in certain regions, e.g., adjacent to the corpus callosum at the cavum septum pellucidum and adjacent to the ventricles. These MØ were W3/25$^+$ (Fig. 1*C*) and LC$^+$. Microglia with short processes also expressed these Ag, but most mature microglia were only weakly W3/25$^+$ or LC$^+$.

Local cortical injury to adult rats evoked a large cellular infiltrate within the lesion site itself and a large number of these were W3/25$^+$ and LC$^+$ monocytes (Fig. 1*D*). In addition, cells with the morphology of mature microglia were W3/25$^+$ at 3 d after injury, increasing in staining intensity after 5 d. It is unlikely that these were recruited cells, but this cannot be ruled out on morphologic grounds alone. The enhanced staining of these reactive microglia was not selective for W3/25 Ag, since LC staining was also increased after injury. However, specificity of staining for mononuclear phagocytes was maintained; W3/25 was not detected on neurons or other glia. Astrocytes, revealed by glial fibrillary acidic protein staining, were clearly distinguishable from W3/25$^+$ reactive microglia. Staining on reactive microglia was still evident 10 d after injury, the latest time examined.

Previous studies in the mouse (1) have shown that the type 3 complement receptor Ag Mac-1 can be detected on mature microglia, but is not present on the surface of resident tissue MØ in bone marrow or on Kupffer cells. In the rat the CR3 Ag OX42 was strongly expressed on MØ in the choroid plexus (Fig. 2*A*) and, unlike W3/25, was also readily detectable on mature microglia in grey matter (Fig. 2*B*) and in the white matter. Although CR3 is not unique to the MØ, OX42 did not label any other cell type in the nervous system of the normal rat and because of its high level of expression in the CNS, the OX42 Ag was an

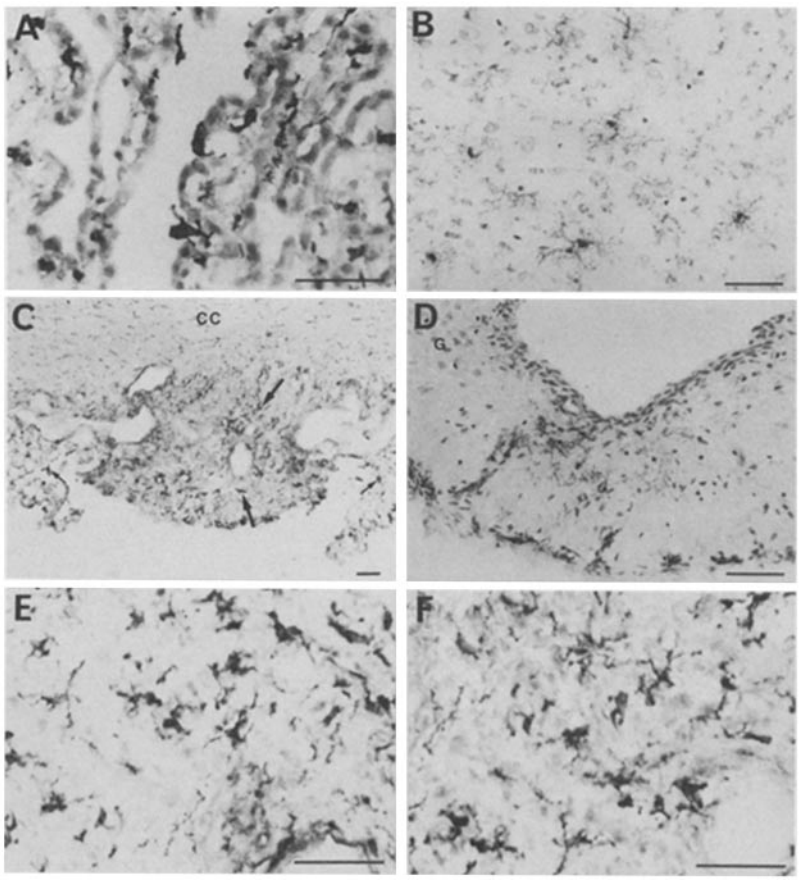

FIGURE 2. (*A*) OX42$^+$ cells in the choroid plexus. (*B*) OX42$^+$ microglia in cortical grey matter. Note the regular spacing of the stained cells and the presence of stained processes. (*C*) W3/25$^+$ cells in subfornical organ lying ventral to the corpus callosum (*cc*). The cells are intensely stained in the subfornical organ (*large arrows*) and adjacent choroid plexus (*small arrows*) but microglia in corpus callosum are not visible as they are only weakly stained. (*D*) Median eminence with W3/25$^+$ cells restricted to the most central position of the field. Microglia in the adjacent grey matter (*G*) were not stained. (*E*) OX42$^+$ cells in the subfornical organ. Note the microglia-like morphology. (*F*) W3/25$^+$ cells in the subfornical organ from a section close to the field shown in *E*. The similar morphology and density of the cells stained in *E* and *F* is evident as is the similar intensity of the staining. Scale bars = 50 μm, counterstained with cresyl violet.

excellent marker for MØ and microglia. These observations also indicated that OX42 and W3/25 Ag were differentially regulated on mature microglia. Unpublished observations by Crocker and Henwood in our laboratory have indicated that W3/25 Ag levels on rat peritoneal MØ can be modulated in cell culture by serum. To examine whether resident microglia fail to express W3/25 Ag because of the blood brain barrier excluding a potential plasma protein inducer, we compared W3/25 and OX42 Ag in regions of the CNS outside the blood brain barrier, e.g., in the subfornical organ and the median eminence. Fig. 2, *C–F* show intense and selective staining of W3/25 Ag on microglia in the subfornical organ and median eminence, comparable to staining with OX42, and similar results were obtained in other regions outside the blood brain barrier

such as the pituitary gland (not shown). Fig. 2, *E* and *F*, show the typical microglial morphology of cells labeled with both these Ab in the subfornical organ, unlike the differential labeling found in grey matter within the blood brain barrier (Figs. 1*A* and 2*B*). LC Ab also stained cells with microglial morphology in regions outside the blood brain barrier, but the enhancement while present was not as great as that found for $W3/25^+$ (not shown).

Our experiments show that in normal rat brain, CD4 Ag was expressed by resident MØ associated with leptomeninges and CSF and by a population of microglia outside the blood brain barrier. The Ag was downregulated during differentiation into mature microglia within the blood brain barrier. After injury, numerous $CD4^+$ monocytes were recruited locally and, in addition, reactive microglia reexpressed CD4. The local environment must therefore play an important role in modulating CD4 expression on MØ. Although not unique to CD4 Ag, the downregulation on mature microglia can be selective, as shown by our studies with OX42 and earlier studies with F4/80 and FcR (1). Our observations may explain, in part, why some other monocyte Ag have not been detected in microglia. Further studies are needed to define the role of the blood brain barrier in regional control of Ag expression.

In our studies we did not observe CD4 surface labeling on any cell type within CNS other than MØ, microglia, or rare lymphocytes. Discrepant findings between these and other studies (8) could be due to variations in mAb and immunocytochemical techniques, especially fixation, or species differences, since MØ express relatively high levels of CD4 in rat, but not in mouse, where it is undetectable (9). It has also been reported that brain contains distinct 3.7- and 2.7-kb mRNA species that hybridize with a CD4 probe (5). The normal sized mRNA can be accounted for by MØ, but not the smaller species (12), and it remains to be shown that this can be expressed as authentic, functional CD4 by any cells within the CNS.

Our findings that monocytes, MØ, and microglia are able to express CD4 in brain are consistent with the hypothesis that these cells could introduce, harbor and disseminate HIV within the CNS. Their blood origin, widespread distribution in white and grey matter, and proximity to the subarachnoid and ventricular spaces in the normal host, together with increased recruitment and CD4 expression during development and after local injury favor such a role. Moreover, these cells express plasma membrane receptors for Ab (1) and complement, for example the CR3 OX42 Ag, which could enhance entry and replication of HIV in MØ via alternative, CD4-independent pathways (10). Although immune enhancement of HIV replication in brain or other MØ has not been demonstrated, the presence of poorly neutralizing Ab in most patients would be likely to favor persistent infection, especially in resident and inadequately activated MØ, resulting from T lymphocyte failure. Whether the consequent production by infected MØ of mediators such as IL-1 and TNF/cachectin could account for dysfunction and loss of neurons and other cells in the CNS remains to be demonstrated.

Summary

Mononuclear phagocytes which express the HIV entry receptor CD4 have been implicated as possible sites of virus replication in brain, but there is still

considerable uncertainty as to which cells in the CNS express CD4 Ag. Although it is not susceptible to HIV infection the rat provides a model to define expression of the CD4 Ag on MØ in brain. We report that the CD4 epitopes W3/25 and OX35 are found only on monocytes, MØ, microglia, and occasional lymphocytes and not on neurons, other glia, or endothelium. CD4 Ag levels are modulated during microglial differentiation, after reactivation after local inflammation, and within the intact blood brain barrier. MØ and microglia also express other potential plasma membrane binding and entry sites for HIV viz Fc and complement receptors that are regulated independently of CD4.

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