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ENTRY OF MONOCYTES INTO THE BRAIN AFTER INJECTION OF CORYNEBACTERIUM PARVUM

by

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ABSTRACT

The receptiveness of the brain to monocyte infiltration was studied in rats that had been injected intracerebrally with Corynebacterium parvum. 0-17 days after intracerebral injection and 18 hours after intravenous injection of diI-labelled isogenous mononuclear cells, host rats were sacrificed and cells from the vicinity of the the injection site and from the contralateral cerebral hemisphere were dissociated and analyzed by flow cytometry. In rats sacrificed 4-11 days post injection, diI-labelled mononuclear cells were detected in cell preparations from the hemisphere ipsilateral and, to a lesser extent, contralateral to the injection site. No extravasation of cells from the blood to the brain was detected in rats injected intracerebrally with saline. By immunohistochemistry, many macrophages were detected in the hemisphere ipsilateral to injection of Corynebacterium parvum. In additional experiments, the dissociated CNS cell population was labelled with OX-42 antibodies to the type 3 complement receptor which is present on monocytes but not lymphocytes. Some cells in the brain were labelled with both diI and OX-42 and therefore were identified as monocytes that had entered the brain from the blood. In conclusion, monocytes can home to both sides of the brain after unilateral injection of a strong inflammatory agent but monocyte infiltration into the brain is delayed in comparison to monocyte inflammatory responses in non-neural tissues.

RÉSUMÉ

La sensibilité du cerveau à l'infiltration de monocytes a été étudiée chez le rat adulte après injection unilatérale intracérébrale de Corynebacterium parvum (C. parvum). Les rats sont sacrifiés entre zéro et 17 jours après injection de C. parvum, et 18 heures après injection en intraveineuse de cellules mononucléaires isogènes marquées avec le DiI. Les cellules provenant du site d'injection et celles provenant de l'hémisphère contralatéral sont alors dissociées et analysées à l'aide de la technique de cytométrie par flux. Chez les rats sacrifiés entre 4 et 11 jours, des cellules marquées au DiI sont détectées dans les préparations cellulaires provenant de la région ipsilatérale, et, dans une moindre mesure, du coté contralatéral au site d'injection. Par contre, aucune extravasation de cellules provenant du flux sanguin n'a été détectée chez les rats ayant subis une injection intracérébrale de tampon salin. Quelques macrophages ont été mis en évidence par immunohistochimie dans la région ipsilatérale au site d'injection de C. parvum. Des expériences additionnelles ont permis de marquer une population de cellules du SNC à l'aide d'un anticorps (OX-42) dirigé contre le récepteur complémentaire de type 3. Quelques cellules provenant du cerveau présentent un double marquage; à la fois au OX-42 et au DiI. Ce sont des monocytes provenant de la circulation sanguine qui ont

pénétré dans le cerveau. En conclusion, les monocytes ont la capacité de pénétrer dans le cerveau, ipsi- et contralatéral au site d'injection d'un agent inflammatoire. Cependant, leur vitesse d'infiltration est plus lente que celle des monocytes pénétrant des tissus non-neuronaux.

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ABBREVIATIONS

Ab	antibody
BBB	blood brain barrier
BMVEC	brain microvascular endothelial cells
C.Parvum	corynebacterium parvum
CNS	central nervous system
CR3	complement type 3 receptor
EAE	experomental allergic encephalitis
HEV	high endothelial venule
HIV	human immunodeficiency virus
ICAM	intercellular adhesion molecule
IFN	interferon
IL	interleukin
LAD	leukocyte adhesion deficiency
LPS	lipopolysaccharide
MAdCAM	mucosal addressin cell adhesion molecule
MCP	macrophage chemotactic peptide
MHC	major histocompatibility complex
MS	multiple sclerosis
PSGL	P-selectin blycoprotein ligand
SIV	simian immunodeficiency virus
TGF	transforming growth factor
TNF	tumour necrosis factor
VCAM	vascular cell adhesion molecule

LITERATURE REVIEW

I. Origin of microglia and responses to injury

Microglia were first described by Rio Hortega (1919; 1932) early this century. He invented a silver carbonate stain with which he could visualize these cells in their entirety. He concluded that they were the "reticulo-endothelial system in the central nervous system". He also believed that the microglia were of mesodermal origin. A variety of theories have been proposed focusing on the origin of microglia in the past 60 years (Ling, 1981). A lot of information has accumulated to support their monocytic origin.

A. Monocytic origin of microglia

In order to prove that microglia of the CNS parenchyma are part of the mononuclear phagocyte system, the following criteria should be satisfied:

a. Originating from bone marrow, the cells move into the CNS and take on the morphology of microglia.

One way to show that a cell comes from the bone marrow is to use chimeras. In this procedure an animal of one strain is completely irradiated to destroy the stem cells of the bone marrow. The animal is then reconstituted with bone

marrow from another strain of the same species, but in which the leukocytes express different markers from those of the host animals. Then, the donor-derived bone marrow cells can be detected in the host animals at different time after reconstitution.

Hickey and Kimura(1988) did the first study using chimeras to show the monocytic origin of microglia. Using immunocytochemical detection of different host and donor MHC antigens, they demonstrated that both perivascular macrophages and microglia within the parenchyma were of donor origin. A similar study also using MHC class I antigens reported that macrophages in the meninges and choroid plexus were also of donor origin(Matsumoto & Fujiwara, 1987).

A potential problem involving the use of MHC for studying the origin of microglia is that the MHC antigens are highly regulated in the CNS microenvironment. To avoid this problem, de Groot et. al. (1992) examined the origin of microglia using a transgenic mouse bearing multiple copies of a bacteriophage gene. They reconstituted the one-day-old and three-month-old animals. The animals were left for up to one year after reconstitution in the former group and 22 months in the latter. The donor cells from the transgenic animals were detected using in situ hybridization for the bacteriophage gene. Around 19% of the microglia in the white matter were double labelled with an Ab to microglia and an in situ probe for the bacteriophage gene, but none in

the gray matter. The authors appeared to have technical problems in detecting microglia in gray matter although there are more microglia in the gray matter than in the white matter (Lawson et al., 1990).

Overall, basing on the data from chimera studies, it can be demonstrated that microglia are derived from the bone marrow.

b. It should be possible to show histologically the morphological transition from the monocytes to microglia in the CNS, if microglia are derived from monocytes.

Ling and coworkerss studies (Ling and Wond, 1993) showed that the monocytes moved into the corpus callosum where they became ameboid microglia and subsequently differentiated to microglia. In the embryonic mouse CNS cells labeled with F4/80, a macrophage specific antibody, have a round morphological look. They are found mainly close to big blood vessels and in white matter. In the later stages of development, cells stained by F4/80 have increasing complex morphology in both white and gray matter.

A quantitative study by Wu et al. (1992) provided support for the continuity of morphology from rounded monocytes to arborized mature microglia. These authors also reinforced the fact that not all the monocytes transform to microglia but many go through cell death. The small number of dying cells seen at one time is only a reflection of the fact dying cells are only visible for a short time and are

cleared very rapidly. Leong and Ling (1992) labeled the rounded macrophages in the corpus callosum by injecting dye intraperitoneally in the neonate rats. If these animals are allowed to survive for 40 days after the injection the cells have the morphology of microglia. Since the dye was rapidly cleared from the circulation the cells must have Been labeled at the time of injection. Those observations on the morphological transitions found in laboratory animals are also true in human fetal brain (Hutchins et al., 1990).

c. If microglia are derived from monocytes, they should express some antigens partially or wholly restricted to phagocytic system.

One of the reasons for the long debate on the origin of microglia is that it has been very hard to demonstrate that microglia and other cells of phagocytic system share antigens. With the appearance of some new antibodies, improved methods of fixation and antibody detection , it is clear that microglia share many antigens with the macrophages. In addition to the macrophage marker F4/80, murine microglia are known to express antigens characterizing leukocytes (the leukocyte common antigen), molecules of known function expressed by monocytes and macrophages (Fc and CR3 recptors), and others (Perry et al., 1985).

B. Distribution of Microglia

The distribution of microglia is heterogeneous. There is a more than five-fold variation in their density and there are more microglia in grey matter than white matter. Areas with higher densities include the hippocampus, olfactory telencephalon, the basal ganglia and substantia nigra. Intermediate densities are found in the cerebral cortex, thalamus and hypothalamus (Lawson et al., 1990). The brain stem and cerebellum have the lowest density. There was no correlation between the distribution of microglia and any special features of the CNS such as neurotransmitter and capillaries.(Lawson et al., 1990)

In the corpus callosum the number of microglia was found to be 5% of all the glia (Perry et al., 1985). In the cerebral cortex the microglia represent about 5% of the total neuronal and glia population (Perry et al., 1985). Lawson et al.(1990) estimated that there were about 3.5 million microglia in the mouse brain. Another study estimated that microglia in the normal human brain were 10% of glia in the grey matter and 8% in the white matter (Akiyama & Mcger, 1990).

C. Receptors and surface antigens

a. CR3

Complement type 3 receptor (CR3) is involved in monocyte/macrophage adhesion, migration and phagocytosis

(Beller et al., 1982 ; Larson et al., 1990). This molecule is a member of the β2 -integrin family. The ligands for CR3 include the iC3b of complement and ICAM-1 found on endothelial cells. The former are involved in opsonization and the latter in the adhesion to and migration of leukocytes across endothelium.

CR3 is detectable on macrophages and microglia of the developing and adult murine CNS (Perry et al., 1985). The level of expression of CR3 is higher on microglia than on some other tissue macrophages (Perry et al., 1989) and its expression is rapidly upregulated in pathological states (Graeber et al., 1988).

b. MHC antigens

Resident tissue macrophages express MHC class I and class II antigens in different amounts. In normal rodent brain, there is no MHC class I or II expression on the resident microglia (Hart & Fabre, 1981). In the CNS of normal animals, MHC class I is expressed on the endothelium but not microglia, and only a few scattered microglia in the white matter express MHC class II (Streit et al., 1989). These antigens are upregulated in different models of injury. The expression of MHC class II in human brain appears to differ from that in the laboratory rodent. In normal white matter of human brain, microglia express readily detectable levels of MHC class II (Hayes et al., 1987).

D. Opposing functions of microglia: protective vs cytotoxic role

Resident microglia play a part in tissue repair after injury, similar to that of resident macrophages in peripheral organs. Being a member of the phagocytic system, they destroy invading micro-organisms, clear deleterious cellular debris, and promote tissue repair by secreting wound-healing factors.

Cytotoxic effects contribute to the function of microglia as tissue guardians in the brain (Banati, 1993). They act as brain phagocytes removing tissue debris. Microglia express receptors for Fc complement on their surface. Especially, during antibody-mediated demyelination , microglia can lyse antibody -coated target cells via interaction of Fc and complement receptors. Activated microglia are capable of releasing several cytotoxic substances in vitro, such as free oxygen intermediates, NO, proteases, arachidonic-acid derivates, excitatory amino acids, quinolonic acid and cytokines (Banati, 1993). TNF-α produced by microglia can cause bystander damage during demyelination. Free oxygen radicals produced by microglia have a neurotoxic effect in co-culture of neurons and microglia. HIV-infected mononuclear cells produce low molecular weight neurotoxins which would cause neuronal damage via NMDA receptor(Giulian et al., 1990). However,

most of the information on the cytotoxic properties of activated microglia are in vitro observations and still remains to be confirmed in vivo.

Microglia also play a protective role. Giulian and colleagues have concentrated on the production of IL-1 and other potential astrocyte growth factors secreted by ameboid microglia in culture (Giulian and Lachman, 1985; Giulian et al.,1986; Giulian et al., 1986). They have shown that IL-1 secreted by microglia from neonatal brains induce astrocyte proliferation by upregulating NGF expression both in culture and in vivo(Giulian and Young, 1988; Giulian et al., 1988). IL-1 may also induce angiogenesis. TGF-β1 produced by activated microglia could promote tissue repair by reducing astrocytic scar formation. Microglia produce both the urokinase-type plasminogen activator and plasminogen (Nakajima, et al., 1992). The observation that plasminogen promotes neurite outgrowth in vitro (Nakajima, et al., 1992) suggests a role for microglia in tissue remodelling.

Thus, microglia have double roles in the CNS: under conditions involving cell death , they work as scavengers clearing debris; in other more subtle injuries they play a surveillance and protection role.

E. Response of macrophages and microglia to CNS injury

In peripheral tissues, mononuclear phagocytes play an

important part in tissue repair following injury. In CNS, as was well recognised by Rio Hortega(1932), microglia respond to almost any kind of insult or injury to the CNS. Although they are downregulated under normal conditions, they respond rapidly to different injuries by changing their morphology and antigen expression. The focus of these studies is the issue of whether it is resident microglia or recruited monocytes which respond to various lesions. In open wounds, there is little doubt that monocytes are recruited to the brain (Perry, 1994). In Wallerian degeneration or retrograde responses, the precise contributions of resident and recruited cells are unclear. In other tissues there is evidence to suggest that resident tissue macrophages play a primary role in injury and infection, also the resident cells are required for the initiation of an inflammation response (Gordon, 1986). If the resident cells are required to initiate an inflammatory response, then the quiescent nature of microglia may be one of the reasons why monocyte recruitment to Wallerian degenerations is slower in the CNS than the PNS.

How do these monocytes enter the CNS under pathological conditions? What important molecular signals and adhesion molecules do they require and how are these signals modulated in CNS? In what pathological processes do these cells participate? Next, I will first discuss the trafficking of monocytes and other leukocytes in non-neuronal tissues, and then the recruitment of different

types of leukocytes to CNS under normal and pathological conditions.

II. Recruitment of leukocytes to inflamed tissue

Circulating and migrating, the white blood cells are able to survey the tissues for infectious pathogens and accumulate at sites of infection and injury. Lymphocytes continually patrol the body for foreign antigen by recirculating from blood, through tissue, into lymph, and back to blood. Granulocytes and monocytes can not recirculate, but escape from the blood in response to signals on the surface of blood vessels. Lymohocytes can similarly accumulate in response to inflammatory stimuli. The nature of the inflammatory stimulus determines whether lymphocytes, monocytes, neutrophils, or eosinophils predominate.

Recent findings show that the traffic signals for lymphocyte recirculation and monocyte or neutrophil accumlation at site of infection are similar. At least three steps with multiple molecular choices at each step, are crucial for leukocyte accumulation at sites of inflammation (Springer, 1994). The important molecules involved in these steps are: selectins, chemoattractants, and integrins and their ligands in the immunoglobulin family. Selectins are responsible for the initial tethering of a flowing leukocyte to the vessel wall. Tethering brings leukocytes into

proximity with chemoattractants that are released from the endothelium cells of the vessel wall. Chemoattractants bind to receptors on the surface of leukocytes. These couple to G proteins, which release signals that activate integrin adhesiveness. The integrins can then bind to their ligands which will finally bring to rest the rolling leukocytes. The different response to infection of leukocytes can be explained by different affinity to distinct combinations of molecules (Springer, 1994).

A. The functions of leukocyte classes correlates with circulatory behaviour

Neutrophils are the most abundant leukocytes in the bloodstream. They are the first to appear at sites of bacterial infection or injury, with the peak of emigration occurring within several hours after the onset of inflammation. Neutrophils are produced at the rate of 10^9 cells/kg body weight /day in the bone marrow and have a half-life in the circulation of 7h. Their life span after extravasation is hours or less. Their primary function is to phagocytose and eliminate foreign micro-organisms and damaged tissue.

Monocytes are far less numerous in the blood than neutrophils, where their half-life is about 24h (Issekutz et al., 1993). They, together with the lymphocytes, become the most abundant in the inflammatory sites within 12-24 hours.

After extravasation, monocytes may also differentiate into longer-lived tissue macrophages such as the Kupffer cells of the liver, which have a half-life of weeks to months.

In contrast to the neutrophil and monocyte, a lymphocyte may emigrate and recirculate many thousands of times during its life history (Springer, 1994). Recirculation of lymphocytes correlates with their role as antigen receptor-bearing surveillance cells. Lymphocytes function as the reservoir of immunological memory, and recirculate through the tissues to provide systemic memory.

B. Endothelium

The endothelium is the most active player in controlling leukocyte traffic because of displaying specific signals. Vascular endothelium is diversified at a number of levels. Large vessels differ from small vessels and capillaries, venular endothelium differs from arterial endothelium, and endothelial phenotype varies between tissues. The migration of leukocytes from postcapillary venules is related to shear stress, which is lower there, and also related to molecular differences on the endothelial surfaces (Nazziola and House, 1992). In agreement with this, P-selectin is much more abundant on postcapillary venules than on large vessels, arterioles, or capillaries (McEver et al., 1989). And expression of E-selectin and VCAM-1 in inflammation is most prominent on postcapillary venules

(Bevilacqua, 1993).

Inflammatory cytokines strongly and selectively modulate the expression of adhesion molecules on endothelial cells. TNF and IL-1 increase the adhesiveness of endothelium for neutrophils and lymphocytes (Pober and Cotran, 1990). IL-4, synergistically with other molecules, increases the adhesiveness of endothelium for lymphocytes and induces VCAM-1. The precise mixture of different cytokines produced at the inflammatory site determines which type of leukocyte will dominant. Thus injection into skin of IL-1 induces emigration of neutrophils and monocytes, such as LPS and TNF-α , INF-γ induces emigration of only monocytes but not neutrophils (Issekutz et al., 1993).

C. Signals and receptors for leukocyte traffic

Over the last 10 years, a number of molecules which mediate leukocyte adhesion have been identified. These adhesion molecules, mediating the three major steps of leukocyte emigration, have been grouped into 4 families based on cDNA sequence homology: selectins, chemoattractants, integrins and Ig superfamily. Members of the same families tend to have similar structure and also exhibit similar function (Springer, 1990).

a. Selectins

The selectin family comprises 3 molecules designated P-

selectin, E-selectin and L-selectin. Each is a single chain glycoprotein, with a N-terminal domain homologous to Ca++-dependent lectins where ligand interactions are mediated (Springer and Lasky, 1991). Consistent with their lectin-like N-terminal domains, each of the 3 selectins recognize counter-structures containing sialylated carbohydrate residues. Selectins are found on endothelium, platelets and leukocytes. They mediate the tethering of flowing leukocytes to the vessel wall through labile adhesion that permit leukocytes to roll in the direction of flow (Lawrence and Springer, 1991).

(a). E-selectin

In 1985, E-selectin was showed to mediate the activation of endothelium by IL-1, TNF and bacterial endotoxin to increase the adhesion of isolated neutrophils (Bevilacqua et al., 1985; Gamble et al. 1985). Subsequent studies demonstrated that E-selectin was also involved in adhesion of monocytes and a subpopulation of T-lymphocytes to cytokine-activated endothelial cells (Picker et al., 1991; Carlos et al., 1991).

E-selectin expression is largely restricted to activated endothelial cells. Maximum expression of E-selectin of cultured endothelial cells was observed at 4-6 hours after exposure to Il-1, TNF and endotoxin (Bevilaqua et al., 1987), followed by a decline and return to baseline by 24-48 hours.

(b). P selectin

P-selectin is a transmembrane protein of around 140 kDa that is stored in the Weibel-Palade bodies of endothelial cells and the granules of platelets. In response to mediators of acute inflammation, such as thrombin or histamine, P-selectin is rapidly mobilized to the plasma membrane to bind neutrophils and monocytes (Bevilacqua, 1993). Recently Sato et al. have described a 220kD sialylated homodimer, designated PSGL-1, that acts as a ligand for P-selectin on leukocytes(Sako et al., 1993). They also suggested that there was a overlap between ligands capable of binding P- and E-selectin, as E-selectin bound to PSGL-1 too.

(c). L-selectin

L-selectin is constitutively expressed by all circulating leukocytes, except for a subpopulation of lymphocytes. Although the initial studies were focused on its role in lymphocyte homing to secondary lymphoid tissues(Gallatin et al., 1983), it has been showed that this molecule is also involved in the adhesion of leukocytes to endothelium in inflammation (Watson et al., 1991). The endothelial ligand for L-selectin in inflamed tissue is not yet established.

b. Chemoattractants and Their Receptors

Chemoattractants are important activators for integrin adhesiveness and they are also involved in directing the migration of leukocytes. Leukocytes move in the direction of the chemoattractants, which diffuse away from the site of its production, where the concentration is the highest (Devreotes and Zigmond,1988).

Classical leukocyte chemoattractants act broadly on neutrophils, eosinophils, basophils, and monocytes. Two subfamilies of chemokines have been defined by sequence homology and by the sequence around two cysteine residues. The CXC or α chemokines, such as IL-8, tend to act on neutrophils and nonhemotopoietic cells involved in wound healing. The CC or β chemokines, such as MCP-1, tend to act on monocytes, eosinophils and lymphocyte subpopulations.

Chemoattractant receptors are G protein-coupled receptors that span the membrane seven times. Ligand binding to the receptors is coupled to exchange of GTP for GDP bound G protein and result in activation of signaling effectors (Wu et al., 1993). Cloning of the receptors for formylated bacterial peptides, C5a, and platelet activating factor has shown that they are expressed on both neutrophils and monocytes, and the receptor for IL-8 is expressed only on neutrophils(Gerard and Gerard, 1994). Thus, the specificity of chemoattractants is regulated by the cellular distribution of their receptors.

c.Integrins

The integrin family is a family of widely distributed adhesion molecules (Hynes, 1992), which mediate cell-extracellular matrix and cell-cell binding. Each integrin contains noncovalently associated α and β subunits, with characteristic structural motifs. Thus far, the best molecules for activation of adhesiveness of integrin are the chemoattractants. For example, adhensiveness of Mac-1 and LFA-1 on neutrophils and monocytes is activated by N-formylated peptide and IL-8 (Larson and Springer, 1990). It seems that the activation of adhesiveness of integrins is due a conformational change in integrins.

Four different integrin subfamilies could be distinguished based on the association of β chains (β1, β2, β3 and β7) with different α chains. β1 sub-family is predominantly involved in mediating leukocyte adhesion to extracellular matrix (Hemler, 1990). β1 sub-family is widely distributed on connective tissue cells and mononuclear cells. β2 sub-family is mainly restricted to cells of leukocyte lineage and mediating leukocyte-endothelium adhesion (Larson and Springer, 1990). β3 sub-family are mainly expressed by platelets and endothelial cells, respectively and they may be involved in adhesion of platelets and endothelial cells (Hynes, 1992). Similar to β 2 integrins, the β7 integrins are also restricted to leukocytes. They play an important role in lymphocyte homing (Hynes,1992).

d. IgCAM-Integrin Interaction:

It is now widely accepted that endothelial cell surface IgCAMs, including ICAM-1, ICAM-2, ICAM-3 and VCAM-1, play key roles in adhesion and transmigration of blood leukocytes. They accomplish these tasks through direct binding to leukocyte cell-surface integrins.

(a). ICAM-1 and ICAM-2, ICAM-3 and their Receptors β2 Integrins

ICAM-1, ICAM-2 and ICAM-3 are products of distinct and homologous genes and were initially identified by their ability to interact with LFA-1, which is a member of β2 sub-family (Springer, 1990). ICAM-1 is expressed in abundance on endothelium after several hours of stimulation by IL-1 and TNF. ICAM-1 is also expressed in an inducible manner in a wide variety of other cell types (Springer, 1990). ICAM-2 is constitutively expressed on endothelial cells, where it may be important for leukocyte trafficking in uninflamed tissue (Springer, 1994).

Endothelial cell surface ICAMs contribute to adhesion and transmigration of leukocytes through binding to β2 integrins (Springer, 1990). Neutrophils, monocytes, lymphocytes, and natural killer (NK) cells express LFA-1, which has already been shown to bind ICAMs (Springer, 1990). Neutrophils, monocytes, lymphocytes and NK cells also express Mac-1 and p150,95. ICAM-1 has also been found to bind Mac-1 (Diamond et al., 1991).

During endothelial-leukocyte adhesion events, a lot of factors may stimulate β2 integrin activity. For example, endothelial cells produce and secrete the integrin activator IL-8 in response to IL-1, TNF, and endotoxin. In addition, the binding of E-selectin to leukocyte surface ligand stimulates integrin function (Lo et al., 1991).

The importance of the leukocyte integrins is illustrated in congenital leukocyte adhesion deficiency (LAD) in which they are deficient because of mutations in the common β2 subunit (Springer, 1990). Patients have recurring infections, often fatal in childhood unless they are corrected by bone marrow transplantation. Neutrophils from these patients fail to orient and migrate in response to chemoattractants and are unable to bind to and cross the endothelium at sites of infection, so that pus fail to form. This is a most striking example of the role of adhesion molecules in leukocyte localization in vivo.

(b). VCAM-1 and the β1 integrins

VCAM-1 is inducible on endothelial cells by IL-1 and TNF, with maximal activity reached around 6-12 hours (Rice et al., 1990). It is also expressed in other cell types in a more restricted manner than ICAM-1. VCAM-1 was shown to support the adhesion to endothelium of lymphocytes and monocytes through an interaction with the α4β1 integrin(VLA-4), which is a member of β1 subfamily(Rice et al., 1990). VCAM-1 binds weakly to α4β7 too. Outside the

vasculature, VCAM-1 participates in a lot of lymphocyte adhesive events (Bevilacqua, 1993).

(c). MadCAM-1 and $\alpha 4\beta 7$ Integrin

MadCAM-1 is expressed on Peyer's patch HEV and on other venules mediating lymphocyte recirculation to mucosal tissue (Streeter et al., 1988) through the interaction with $\alpha 4\beta 7$ integrin. MadCAM-1 contains three Ig-like domains and a mucin-like region (Briskin et al., 1993). Carbohydrates attached to the mucin-like region of MadCAM-1 bind L-selectin and mediate lymphocyte rolling (Berg et al., 1993). So, MadCAM acts both as an integrin and a selectin ligand.

D. Adhesion molecules mediating recruitment of monocytes

There are several adhesion molecules expressed on monocytes which are involved in monocyte adhesion to endothelium during physiological and pathological conditions. Among the integrins, fresh blood monocytes express considerable amounts of VLA-4, -5, -6 and three beta 2 integrins, LFA-1, Mac-1, and p150,95 (Patarroyo, 1994). ICAM-1, ICAM-2, ICAM-3 and LFA-3 are all Ig-related molecules expressed by monocytes. VCAM-1 , another member of this family which is found on activated endothelium, is not expressed by monocytes , but these cells express its

receptor, VLA-4. L-selectin is the only selectin expressed by monocytes. On the other hand, E-selectin and P-selectin , expressed on activated endothelium, recognize oligosaccharides such as Lex and sislyl Lex which are found on the monocyte cell surface.

In vitro studies with blocking antibodies have indicated that adhesion of monocytes to vascular endothelial cells is mediated, at least in part, by beta 2 integrins (Beekhuizen, H. and Furth, R. V., 1993). Other adhesion molecules involved in monocye-endothelium interaction include ICAM-1, VLA-4, VCAM-1, L-selectin and E-selectin. Blocking antibodies to L-selectin or Mac-1 inhibit neutrophil and monocyte accumulation in inflamed peritoneum. The latter antibody also inhibits neutrophil and monocyte migration in a delay type hypersensitivity reaction in footpad. Additional in vitro studies with blocking antibodies have demonstrated that most monocyte activities are adhesion-dependent (Beekhuizen, H. and Furth, R. V., 1993). Moreover, beta 2 integrins participate in HIV-induced syncytium formation in monocytic cells (Patarroyo, et al., 1990).

III. Traffic of blood cells into the CNS

The CNS has long been considered to be an immunological privileged site, generally devoid of cells derived from the

peripheral circulation (Wekerle, H. et al., 1986). This view has been based on four arguments: (i) lack of lymphatic drainage; (ii) impaired rejection of transplants; (iii)low expression of MHC class antigens; (iv) the complex BBB to cell extravasation from circulating blood. The blood brain barrier (BBB) is a specialised vasculature consisting of endothelial cells with tight junctions, preventing the entrance of large proteins and leukocytes into the CNS. However, studies have revealed that activated T cell can across into the CNS, at very low levels under normal conditions and much higher numbers during neurological autoimmune disorders like MS or its animal model EAE (Wekerle, H. et al., 1986). Also, the migration of viruses into the brain has been suggested, in the case of HIV, to be mediated by infiltration of infected monocytes through the BBB. Under pathological conditions, the upregulation of cell adhesion molecules on the endothelial cells of BBB is, at least partially, responsible for infiltration of large numbers of leukocytes into the CNS.

A. Endothelial cells and the BBB

The BBB is comprised of the endothelial cells, pericytes, astrocyte foot processes and perivascular microglia cells (Williams and Hickey, 1994). Under normal conditions, the BBB is relatively impermeable to the cells and large molecules from the blood. In response to different

pathological stimuli, both large molecules and circulating cells can cross the BBB and get into the CNS, but the molecular basis for infiltration of cells and large molecules are quite different. Under normal conditions, the expression of many adhesion molecules which are known to express on the endothelial cells in other tissues is low to nonexistent on CNS endothelium. These adhesion molecules are strongly upregulated by inflammatory stimuli. The list of all the molecules is incomplete, but includes ICAM-1, ICAM-2, VCAM-1, LFA-3, CD44, E-selectin and P-selectin (Williams and Hickey, 1994). Unquestionably, the secretion of IL-1, TNF, IL-6 and interferon in the vicinity of a vessel drastically alters the endothelial adhesion profile. T lymphocytes, monocytes/macrophages and some glial cells can produce those cytokines when activated. It has been shown that the BBB can become activated in a variety of CNS diseases where these cytokines are present (Tyor et al., 1992). These cytokines which are elevated in the CNS can stimulate the expression of adhesion molecules necessary for trafficking of leukocytes into the CNS (Pober et al., 1987 ; Lassmann et al., 1991; Wekerle et al., 1991).

B. T cells traffic in multiple sclerosis

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the CNS. Lesions in MS are characterized by the inflammatory cells infiltration, mainly

T cells and monocytes/macrophages. Naive T cells cannot readily penetrate the BBB, but, upon activation, T cells easily enter the CNS (Fabry et al., 1994). Activated T cells enter the CNS regardless of their antigen specificity, but only T cells that recognize CNS antigen stay (Hickey et al., 1991).

Several adhesion molecule-receptor pairs have been shown to be involved in T cell extravasation into the CNS. These include VCAM-1/VLA-4, ICAM-1/Mac-1, and ICAM-1/LFA-1. Studies suggest that VCAM-1/VLA-4 interactions at the BBB might be crucial for leukocyte entry during autoimmune disease. Yednock et al.(1992) demonstrated that treatment of animals with a monoclonal antibody against VLA-4 reduced both the clinical and histological severity of experimental allergic encephalomyelitis (EAE), which is an animal model for MS. VLA-4 expression by proteolipid protein- or myelin basic protein-specific T cells was also shown to be crucial for their ability to enter the CNS, as well as their encephalitogenic potential (Baron et al., 1993; Kuchroo et al., 1993). There is a similar report of the inhibition of the induction of EAE using anti- ICAM-1 monoclonal antibody (Archelos et al., 1993). In addition, infiltration of the CNS with mononuclear cells was dramatically reduced upon treatment with anti-ICAM-1 mAb in rats with active EAE. Further evidence that ICAM-1 is involved in EAE comes from the findings that mAbs against LFA-1 and MAC-1 delayed the onset and diminished the severity of EAE (Gordon et al.,

1995).

Chemokines act in concert with adhesion molecules to recruit inflammatory cells. Ransohoff et al. (1993) investigated the production of MCP-1(chemotactic for monocytes) and IP-10(chemotactic for neutrophils and T cells) in the CNS during EAE. They found that the expression of MCP-1 and IP-10 was correlated with the appearance of clinical and histological EAE. RT-PCR analysis of spinal cord from animals with EAE showed that mRNAs encoding RANTES, MIP-1, IP-10 and MCP-1 were induced 1-2 days prior to clinical signs, and achieved the highest level at disease onset (Godiska et al., 1995).

Thus, adhesion molecules such as VCAM-1 and ICAM-1, and chemokines are important in guiding inflammatory leukocytes into the CNS, contributing to the neuropathology of MS and EAE. Other important molecules involved in this process include members of the selectin family and their ligands. At this time, there is not much information on expression of selectins and ligands in the CNS.

C. Monocyte traffic into the CNS

As a group of cells that can become infected with HIV in the periphery, monocytes/macrophages are of great interest. The infiltration of blood-borne monocyte derived macrophages characterizes HIV induced encephalitis (Price et al., 1988). Secretory products produced by infected brain

macrophages impair the functions of CNS. Up to a third of AIDS patients develop neurological symptoms, including AIDS dementia in most serious cases. The recruitment of mononuclear phagocytes into brain during disease likely controls the progression of CNS disease (Nottet et al., 1996). However, the mechanism of this entry remains unclear. Two theories of viral entry into the CNS have been proposed (Gilles et al., 1995). The first suggests that HIV-1 enters the brain via latently infected macrophages. A second theory is that HIV-1 enters brain tissue by direct infection of brain microvascular endothelial cells(BMVEC). In support of the second theory, a recent report demonstrated that BMVEC are readily infected with lymphocytic HIV-1 (Moses et al., 1993). However, the pathobiologic significance of these findings remains in question because few reports show BMVEC infection in vivo. In agreement with the first theory, Nottet et al. (1996) showed that coculture of infected monocytes with BMVEC resulted in E-selectin and VCAM-1 expression on BMVEC. They also showed that monocyte binding to encephalitic brain tissue was blocked with Abs to VCAM-1 and E-selectin. They concluded that HIV-1 entry into the brain is the consequence of the ability of virus-infected monocytes to induce adhesion molecules on brain endothelium. Similar result came from the study of SIV (simian immunodeficiency virus) infection, in which Sasseville et al., (1994) found ICAM-1 expression on BMVEC in association with virus-infected monocytes.

In other types of inflammation in the nervous system, such as stab wound in the brain, transection of the sciatic nerve, or injection of LPS, C. parvum and other inflammatory stimuli, will cause the accumulation of macrophages in the nervous system (Montero-Menei et al., 1996; Lu and Richardson, 1993). These accumulated macrophages might contribute to the support of neural repair. The signals for the recruitment of macrophages are still unclear. Engelhardt et al. (1994) reported the upregulation of ICAM-1 and VCAM-1 on brain endothelium starting 2 days after C. parvum intracerebral injection. Expression of these two molecules may be essential, although not sufficient, for the recruitment of inflammatory cells into the CNS. The other factors leading to a successful recruitment still need to be defined.

INTRODUCTION

Historically, the central nervous system (CNS) of mammals has been considered to be an immunologically privileged region, poorly accessible to cells in the circulation. Although it is clear now that lymphocytes can enter the brain with appropriate stimulus (Wekerle, et al., 1986; Williams and Hickey, 1995), the ability of monocytes to cross cerebral capilary walls is less well documented. The number of macrophages in the brain does increase under some circumstances, for example after tissue necrosis or strong inflammatory stimulus(Andersson et al., 1992; Montero-Menei et al., 1996; Soares et al., 1995). However, the relative contribution of peripheral blood monocytes and resident microglial cells to macrophage and microglial responses in the CNS has been difficult to ascertain (Fujita and Kitamura, 1976; Ling and Leong, 1988; Perry and Gordon, 1991; Stevens and Bahr, 1993). In studies of otherwise normal brains in radiation chimeric animals, hematogenous replenishment has been documented for perivascular microglial cells but rarely for parenchymal microglial cells (Hickey and Kimura, 1988). The microglial hyperplasia surrounding axotomized motoneurons is thought to arise predominantly from proliferation of resident microglial cells with little cellular infiltration from the blood (Graeber et al., 1989). On the other hand, some extravasation of peripheral blood mononuclear cells has been

documented soon after stab wound to the brain (Imamoto and Leblond, 1977). 2-4 days after injection of LPS into the brain, increased numbers of macrophages are found near the injection site (Andersson et al., 1992). In the latter experiments, it was suggested that most of these macrophages originate from the blood rather than from transformation and/or proliferation of endogenous microglial cells but the evidence was indirect. The majority of macrophages in the brains of rats with experimental allergic encephalomyelitis are blood-derived but the timing of extravasation of these macrophages is unknown (Rinner et al., 1995).

In non-neural tissues, macrophage responses are more rapid than in the nervous system and are associated with much more visible accumulations of lymphocytes and polymorphonuclear leukocytes. Recently, considerable information has been obtained regarding the selectins, integrins, and chemokines that mediate interactions between endothelial cells and leukocytes which lead to extravasation of white blood cells into tissues (Springer, 1994). Also, adoptive transfer techniques have been developed to analyze chronological and molecular characteristics of inflammatory cell extravasation in non-neural tissues (Issekutz and Issekutz, 1995; Issekutz et al., 1981) and T-lymphocytes into the inflamed brain (Wekerle et al., 1986).

In this report, it is documented by adoptive transfer that monocytes can indeed enter the brain during a discrete time interval after a local inflammatory stimulus.

MATERIALS AND METHODS

I. Animals and Surgical Procedures

Male Lewis rats (Charles River Laboratories) weighing 270-310 grams were anesthetized with pentobarbital and injected intracerebrally with 35 μg of C. parvum or PBS (phosphate-buffered saline) in a volume of 2.5 μl. The site of injection was 4 mm lateral to the sagittal suture, midway between the coronal and lambdoid sutures and 4mm deep to the cranium. In a single rat, 8 ml of a 4% solution of thioglycollate were injected intraperitoneally.

II. Preparation and DiI-labelling of donor peripheral blood cell population

Deeply anaesthetized donor male Lewis rats were bled by cardiac puncture. A fraction enriched in peripheral blood mononuclear cells (PBMC) was obtained from diluted whole blood by centrifugation on Ficoll-Paque at 1,100 x g x 30 min. at 22°C. The mononuclear cell fraction was recovered from the gradient, washed to remove all traces of Ficoll, and resuspended at a concentration 1.0-2.0 $x10^7$ cells/ml in RPMI 1640 without fetal calf serum (FCS). Immediately after purification, PBMC populations were labelled with the lipophilic dye DiI (Barron et al., 1990) (Molecular Probes, Inc.) at a concentration of 50μg/ml, for 5min. at 22°C. The

labelling reaction was terminated by diluting the samples in an equal volume of FCS and cells were pelleted by centrifugation at 400 xg for 10 min. Cells were resuspended in RPMI 1640 + 10% FCS, repelleted, and resuspended at a concentration of 3 x 10^7/ml. By morphological criteria, approximately 20% of the nucleated cells in these preparations were monocytes and most of the rest were lympocytes.

III. Adoptive transfer

Approximately 2 x 10^7 cells including 3 x 10^6 monocytes were injected into the femoral vein of host rats that had been injected intracerebrally with C. Parvum or PBS 1 to 16 days previously.

III. Isolation of cells from brain tissue

Approximately 18 hours after injection of DiI-labelled cells, deeply anaesthetized animals were perfused through the aorta with 250ml of PBS. A block of tissue 4 x 4 x 6mm from the injection site and a similar region of the contralateral cerebral hemisphere were removed and mechanically dissociated. The cells were pelleted at 400 x g x 10 min., resuspended in 70% isotonic Percoll and overlaid with equal volumes of 37% Percoll and 30% Percoll. After centrifugation at 450 x g x 20 min., cells were recovered

from the interface between the 70% and 37% fractions of the gradient (Hickey and Kimura, 1988). 0.5 -1.0 x 106 cells were recovered from these blocks of tissue. In the single rat with intraperitoneal injection of thioglycollate, peritoneal cells were recovered by lavage with RPMI.

IV. Flow Cytometry

Aliquots of 105 CNS cells were analyzed using fluorescence activated cell scanner (FACScan) technology using a LYSYS software (Becton Dickinson). For analysis of CNS cell populations, light scatter gates were drawn to focus on brain cell populations having the cell size and granularity profiles of monocytes and granulocytes. In some experiments, purified CNS cell populations were labelled (20 min., 4oC) with fluorescein isothiocyanate coupled mouse anti-rat complement receptor type 3 (MRC OX-42) (Cedarlane) at 1:1000 after blocking with normal mouse sera (15 min., 4oC). These cells were analyzed with filters for diI fluorescence and FITC fluorescence.

V. Immunohistochemistry

Deeply anaesthetized rats were perfused with 120ml of PBS followed by 120 ml of 4% paraformaldehyde. Brains were removed, post-fixed in 4% paraformaldehyde for 30 min. to 1 hr, washed with PBS, and incubated in 15% sucrose overnight

at 4oC. Brain samples were immersed in TissuTek OCT compound (Miles Laboratories) and frozen in 2-methylbutane cooled in liquid nitrogen. Sections approximately 10 μm thick were mounted on glass slides and incubated sequentially with 10% normal goat serum, OX-42 mouse monoclonal primary antibody 1:1000, biotinylated goat anti mouse IgG 1:1000, preformed avidinDH:biotinylated horseradish peroxidase complex (ABC reagent, Vector Laboratories) and 0.05% diaminobenzidine tetrahydrochloride solution(DAB) plus 0.01% H2O2]. The reaction was stopped by washing with water and sections were counterstained with 0.2 % toluidine blue.

RESULTS

I. Mononuclear cells pass from blood to brain after C. parvum injection

A. Isolation of DiI-labelled cells at sites of CNS injury

FACScan fluorescence profiles of total cell population, purified from brain tissue 5 days after C.parvum injection, are shown in Figure 1. Fluorescent signals located above the diagonal in the region designated R1, represent DiI-labelled (red fluorescence, along the Y-axis) cells which have passed from the peripheral circulation to regions of inflammation in the CNS. In this particular sample, 0.13% of the cells collected from the site of C. parvum injection (Figure.1a) were labelled with DiI while 0.03% of the cells isolated from the contralateral side (Figure. 1b) of the brain were positive for the DiI label.

B. Time course of migration of mononuclear cells from blood to brain after C. parvum injection

Following intravenous injection of DiI-labelled mononuclear cells during the first three days after intracerebral injection of C.parvum, the number of diI-labelled cells which could be shown to enter the brain was very small. Counts were not significantly different from

counts in brains of animals without intracerebral injection of C. parvum or without intravenous injection of cells. However, in adoptive transfer studies performed 4-11 days after intracerebral injection, extravasation of diI-labelled cells near the injection site could consistently be documented by FACScanning (Figure. 2). Mean percentages of diI-labelled cells in dissociated cells from injection sites of brains removed 2-3,4, 5-6, 10-11, or 17 days after C. parvum injection were 0.05, 0.16, 0.17, 0.22, and 0.03. A parallel, smaller, statistically significant increase in labelled cells was also detected in the contralateral cerebral hemisphere from 5-11 days.

In 4 studies performed 2-16 days after intracerebral injection of PBS rather than C. parvum, the percentage of labelled cells in the ipsilateral hemisphere did not exceed 0.01.

In a rat sacrificed 24 hours after intraperitoneal injection of thioglycollate and 18 hours after intravenous injection of 2.3 x 107 diI-labelled mononuclear cells, 1.3 x 107 nucleated cells were recovered from the peritoneum of which 0.34% were diI positive.

II. Many of the mononuclear blood cells homing to the injured CNS are monocytes

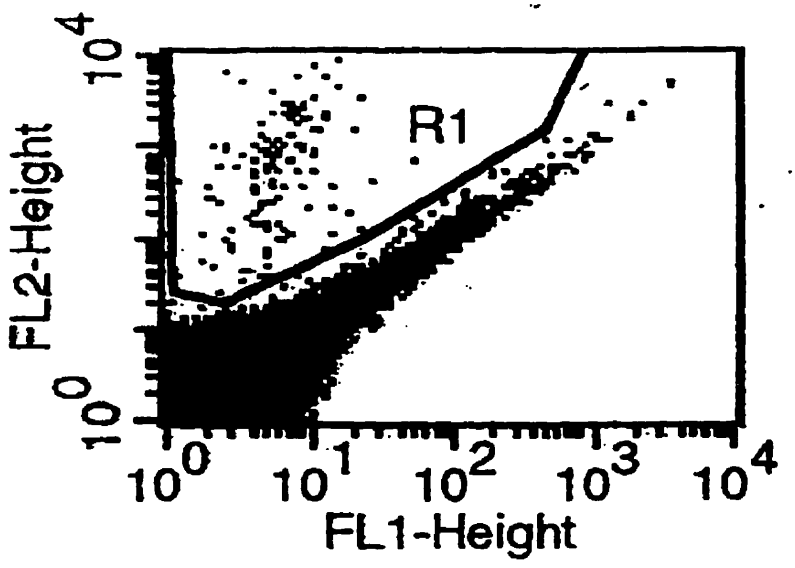
The monoclonal antibody, OX-42, which recognizes the type 3 complement receptor present on monocytes, microglial

cells, and macrophages but not lymphocytes was used to document more precisely the nature of homing mononuclear cells. Brain cell populations were incubated in the presence of FITC-labelled OX-42 antibodies and tightly gated to select cells with size and granularity flow cytometry profiles of monocytes and granulocytes. 36% of these cells, purified from rat brain tissue samples were OX-42 positive. Therefore this purified cell population is highly enriched for microglia and other CNS macrophages. In analysis of cells dissociated from the site of C.parvum injection five days after injury (Figure. 3), 0.35% of the size/granularity selected population were diI-positive and of these 22% were also labelled with the OX-42 antibody. Fewer DiI-labelled cells, 0.24% of the size/granularity selected population, were detected in cell preparations from the contralateral hemisphere. However, the percentage of DiI-labelled cells which was labelled also with the OX-42 antibody was 28%. In a second rat, similar percentages of diI-labelled cells in the ipsilateral and contralateral hemispheres were OX-42 positive. From these results, it is concluded that at least some of the cells that pass from the peripheral circulation to the site of injection and to the opposite side of the brain are monocytes.

III. Immunohistochemical analysis of sites of inflammation in the CNS

Examination of brain tissue sections processed for OX-42 immunoreactivity revealed a pattern of macrophage accumulation in the area of the injury which is consistent with previous reports (Engelhardt et al., 1994; Kennedy et al.,1989). A few OX-42 positive cells were seen near the injection site in brains removed 1 day after injection of C. parvum. Most of this OX-42 positive cell population is located in the region of blood vessels and the ventricles at this early time period(1 day) after C. Parvum injection (data not shown). In brains removed 7 days after injection, many OX-42 positive cells with macrophage morphology were observed in the brain parenchyma, in perivascular spaces and in the subarachnoid space (Figure. 4). In the contralateral hemisphere, OX-42 immunoreactivity was enhanced on many stellate cells which resembled microglial cells but the number of macrophages was not increased conspicuously.

a



b

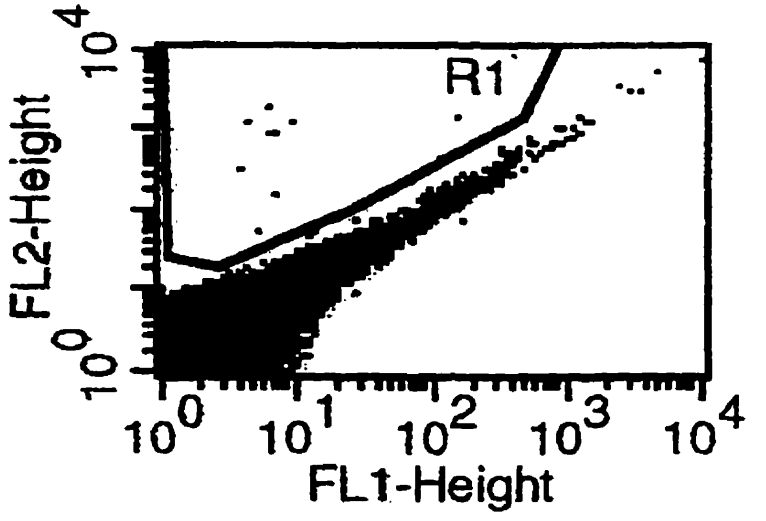


Fig.1 Purification of PBMC from sites of CNS injury

FFACScan profiles of brain cell preparations collected from the site of injury(a) and contralateral side(b) 5 days after C.parvum intracerebral injection. Cells located in the R1 region, above the diagonal, represent DiI positive cells which have passed from the peripheral circulation to the brain.

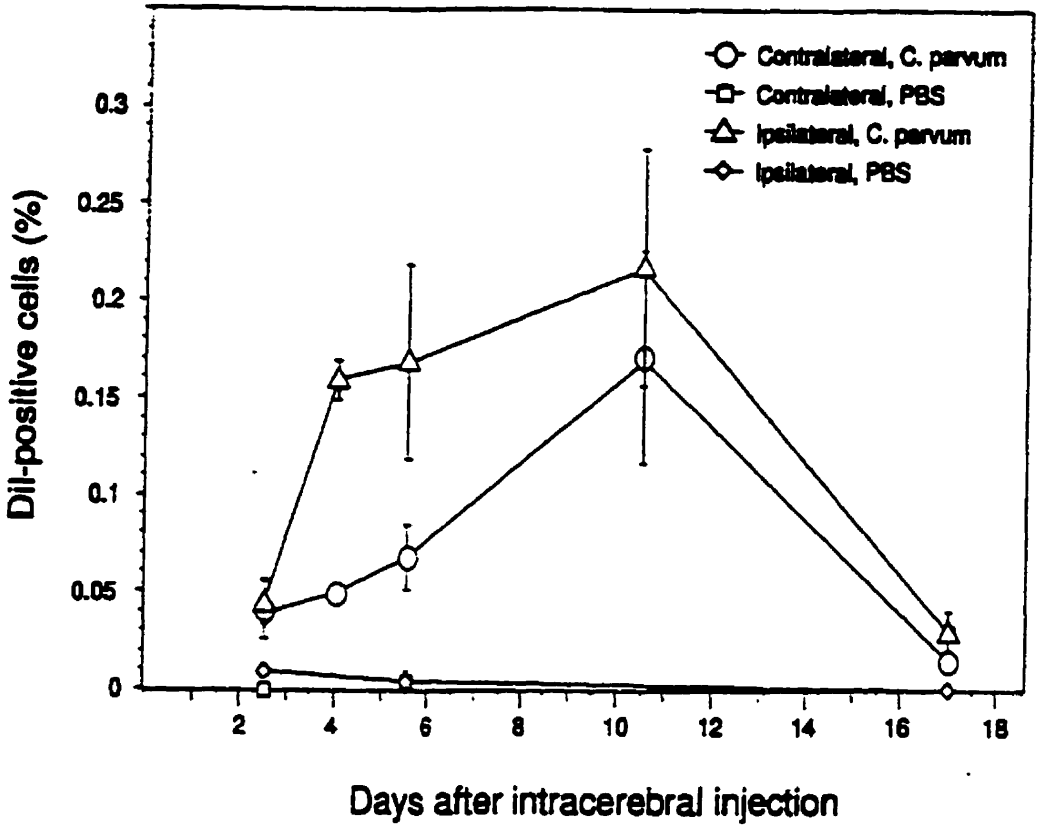


Fig.2 Leukocyte homing after cerebral injection

FACScan data to show the percentage of DiI-labelled cells in cell preparations from the site of cerebral injection or from a similar region of the contralateral hemisphere at several time intervals after cerebral injection of C. parvum or saline and 18 hours after intravenous injection of leukocytes. m ± s.e.m., n=2-12 for C. parvum data.

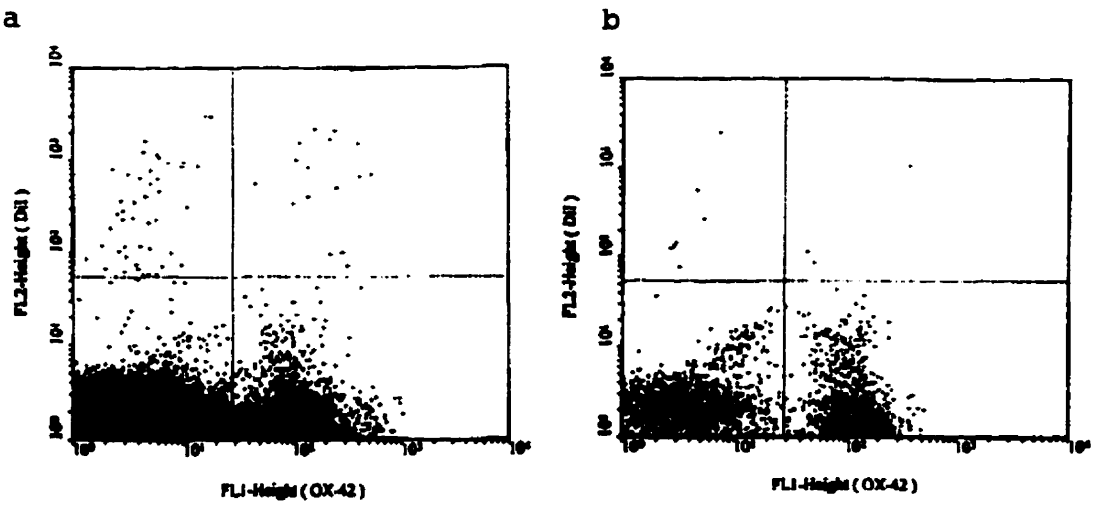


Fig. 3 FACScan analysis of DiI and FITC-OX42 labelled CNS cell population

FACScan profiles from preparation enriches for macrophage/microglia cells taken at the site of injection of C. parvum 5 days later. Fluorescent signals located in the upper left quadrant of the dot plot represent cells positive for the DiI label but not for OX42. Cells in the upper right quadrant are labelled with both DiI and OX42. Cells in the lower right quadrant represent cell populations labelled with OX-42 alone. Fig. 3a and 3b show data from samples collected ipsilateral and contralateral to the site of injection of C. parvum. On both sides, 20-30% of labelled cells are also OX-42 positive.

a

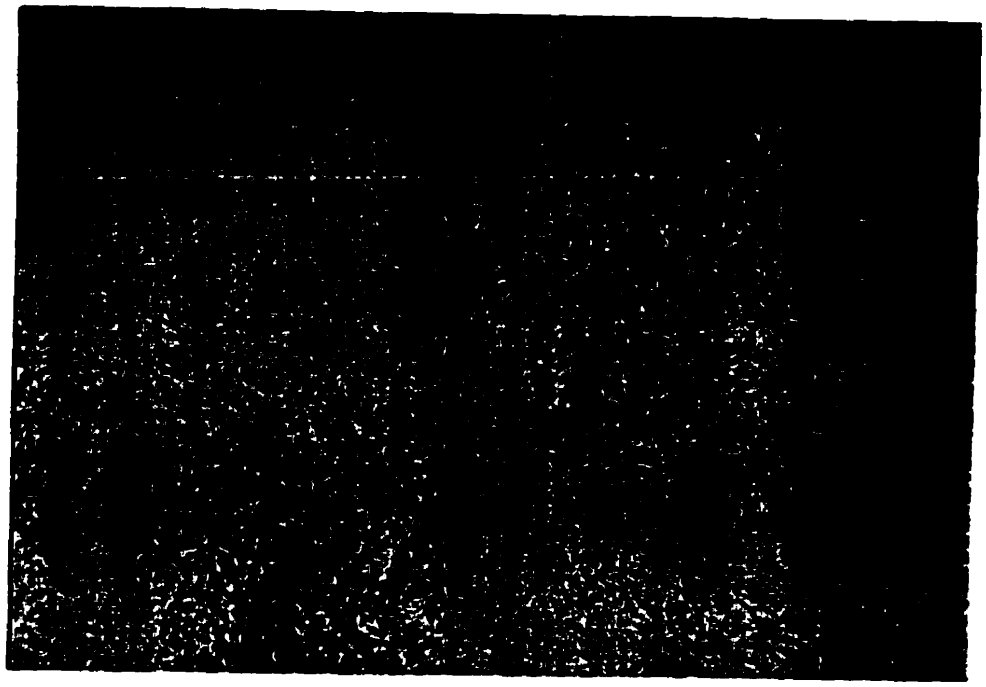


Fig. 4 OX-42 immunohistochemistry

Sections of a brain removed 7 days after C. parvum injection and processed for OX-42 immunohistochemistry. a: numerous OX-42 labelled cells in the parenchyma at or near the site of injection (x80).

b

Fig. 4 OX-42 immunohistochemistry

Sections of a brain removed 7 days after C. parvum injection and processed for OX-42 immunohistochemistry. b: OX-42 labelled cells surrounding blood vessels, in the cortex (right), and in the subarachnoid space (left) (x280).

c

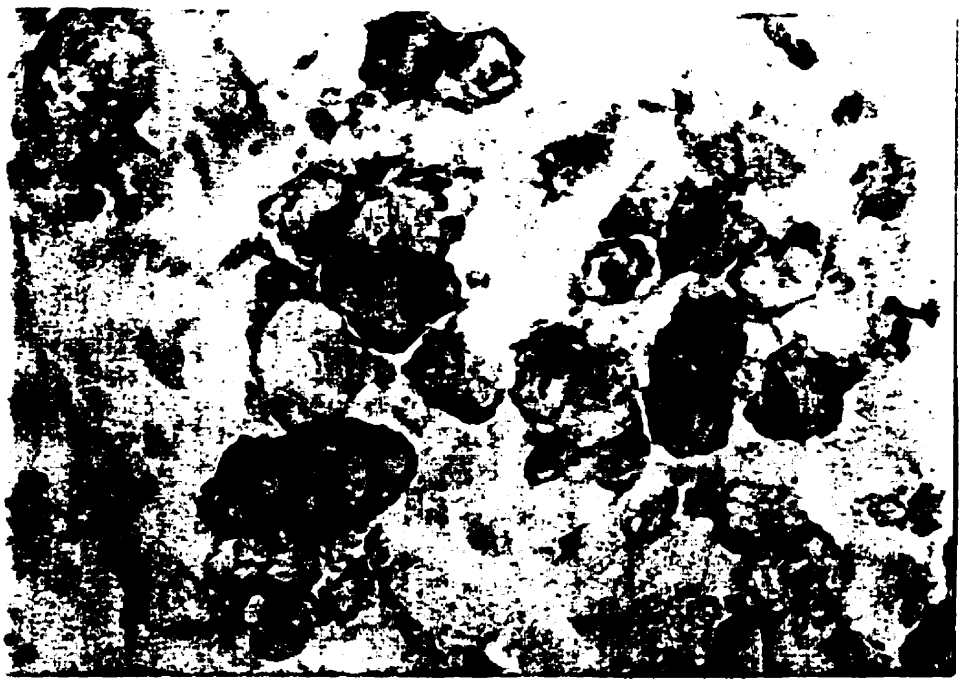


Fig. 4 OX-42 immunohistochemistry

Sections of a brain removed 7 days after C. parvum injection and processed for OX-42 immunohistochemistry. c: higher magnification (x1020) again at the injection site to show that the cellular morphology is that of mactophages.

d

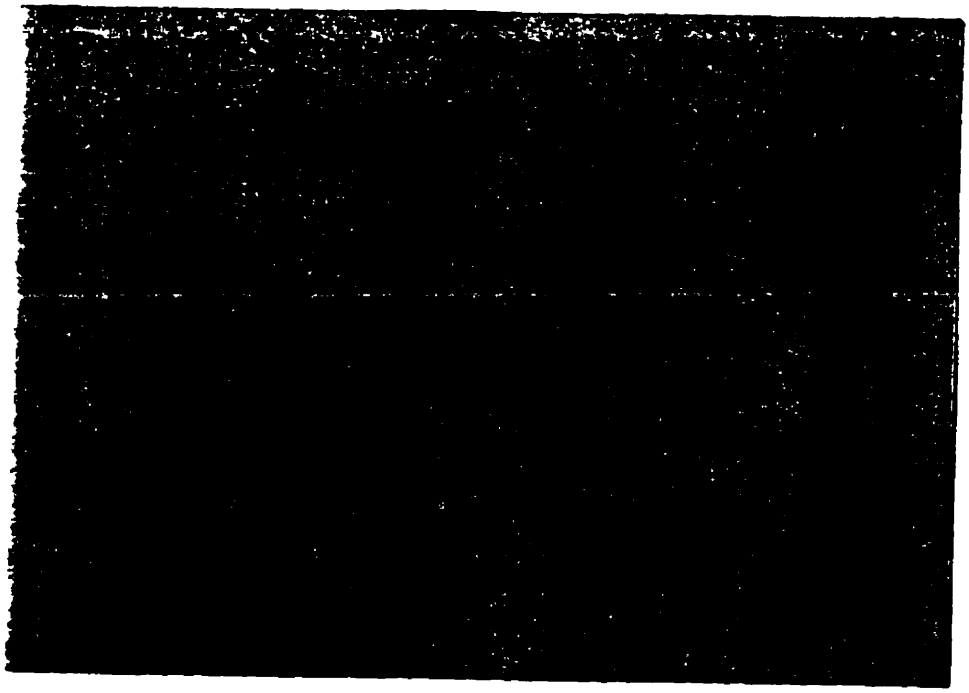


Fig. 4 OX-42 immunohistochemistry

Sections of a brain removed 7 days after C. parvum injection and processed for OX-42 immunohistochemistry. d: enhanced OX-42 immunoreactivity of putative microglial cells in the contralateral hemisphere (x1020).

DISCUSSION

I. Validity of the results

The results support the hypothesis that monocytes home from the blood to the brain following intracerebral injection of the strong inflammatory agent C. parvum. In these adoptive transfer experiments, diI-labelled cells with properties of monocytes were consistently detected by FACscanning of cells dissociated from brains removed 4-11 days after C. parvum injection but not in uninjected brains or brains injected with PBS. The extravasation of hematogenous cells does not represent simple physical disruption of the vascular endothelial cells because it is not associated with extravasation of red blood cells. The results are consistent with earlier histological documentation of a delayed macrophage response to injection of C. parvum (Engelhardt et al., 1994; Barron et al., 1990). The diI-labelled cells that were injected intravenously were a mixture of monocytes and lymphocytes and, in most experiments, the relative numbers of these two cell types entering the brain was not determined. However, in double labelling experiments (diI and OX-42), it was confirmed directly that monocytes can enter both hemisphers of the brain. Because T-cells are found in the brain following C. parvum injection but not in the normal brain, it is assumed that they also home to the brain in response to this

inflammatory stimulus (Engelhardt et al., 1994).

Although the data are not sufficient to quantify how many monocytes enter the brain after C. parvum injection, this number appears to be less than that associated with peritoneal inflammation.

The diI-labelled isogenous cells can be regarded as a tracer population because their total number is smaller than the circulating population of endogenous monocytes and they probably tend to be removed rapidly by the spleen because of cell membrane modifications induced during manipulation ex vivo. In experiments with 51Cr-labelled mononuclear cells, only 30% of the injected radioactivity could be recovered in the blood 2 hours after injection (data not shown). Thus, most of the extravasation of mononuclear cells that was documented during 18 hours following injection of isogenous cells probably occurred within two hours of injection.

II. Responses in the contralateral hemisphere

By adoptive transfer and FACScanning, monocyte extravasation into the contralateral hemisphere was demonstrated unequivocally. The results are consistent with other observations of contralateral macrophage responses to a unilateral neural insult. For example, after peripheral nerve injury, macrophages accumulate in contralateral as well as ipsilateral dorsal root ganglia (Lu and Richardson, 1991).

Although monocyte infiltration into the contralateral hemisphere was documented by flow cytometry, macrophage hyperplasia was not evident by immunohistochemistry. One possible explanation of this discrepancy is that monocyte/macrophages proliferate more actively in the ipsilateral than in the contralateral hemisphere. It is conceivable but unlikely that monocytes are transformed into microglial cells.

III. Possible molecular signals for monocyte infiltration

Molecular events after intracerebral C. parvum injection result in a delayed infiltration of monocytes both locally and at a distance with associated T-lymphocyte (Engelhardt et al ., 1994) but not polymorphonuclear reaction. A delay between injury and appearance of macrophages appears to be characteristic of injury in the CNS and PNS (Andersson et al., 1992; Lu and Richardson, 1991). The response in the contralateral hemisphere is likely to represent molecular changes in endothelial cells rather than a change in monocytes since it is demonstrable for donor monocytes with only brief exposure to any putative systemic stimulus. The unknown signal to endothelial cells in the contralateral hemisphere could be be disseminated hematogenously or parenchymally. The temporal course of the capacity of the brain to attract monocytes that is demonstrated in these experiments correlates well with the

time course of induction of VCAM (vascular cell adhesion molecule) and ICAM (intracellular adhesion molecule) after C. parvum injection (Engelhardt, 1994). VCAM which interacts with α4 integrins and the chemokine MCP-1 (monocyte chemotactic peptide) are of particular interest because they are effective for monocytes but not polymorphonuclear cells. The contribution of individual molecules to endothelial activation and monocyte infiltration into the brain might be assessed by injection of antibodies (Austrup, 1997; Issekutz and Issekutz, 1995, Nakao et al., 1995, Rosen and Gordon, 1987, Yednock et al., 1992) or in mice with null mutation of such molecules (Wilson et al. 1993).

Macrophage reactions surrounding axotomized neurons and in degenerating nerve segments are more pronounced in the PNS than CNS (Barron et al., 1990) and macrophages at both sites in the PNS can contribute to axonal regeneration (Brown et al., 1991; Lu and Richardson, 1991). Manipulation of monocyte/macrophage responses in the nervous system is one possible strategy to enhance repair in the injured CNS.

SUMMARY

Data in this thesis support the hypothesis that monocytes, derived from the peripheral circulation, home to the sites of injury in the CNS following intracerebral injection of C. parvum.

The results of these adoptive transfer experiments suggest that the homing of monocytes from the circulation to sites of injury in the CNS appears to reach a peak at 4-11 days after C. parvum injection. By day 17 after C. parvum injection, the rate of homing by cells from the peripheral circulation to sites of injury in the CNS is low and close to that seen for the first few days after injection. While more cells are seen to home to the site of injury, significant numbers of cells are also seen to accumulate in contralateral regions of the brain.

Immunohistochemistry of brain tissue sections revealed an accumulation of OX-42 positive CNS macrophages/microglia at sites of injury following intracerebral injection of C.parvum. Peak periods of accumulation of OX-42 positive cells at sites of injury are consistent with those shown by adoptive transfer technique.

The kinetics of infiltration of monocytes to sites of CNS injury reported in this thesis, are similar to the kinetics of expression of the ICAM-1 and VCAM-1 class of cell adhesion molecules observed on brain endothelium after C. parvum injection(Engelhardt et al., 1994). The close

correlation between the initial time of detection of the expression of cell adhesion molecules, two days after injury, and the kinetics of infiltration of monocytes to sites of injury, suggests that those cell adhesion molecules might be involved in the homing of monocytes.

In conclusion, monocytes can home to the site of injury following intracerebral C.parvum injection, and to a lesser extent, they can home to the contralateral side too. The homing of monocytes is relatively delayed in CNS as compared to non-neuronal tissues. The infiltration of monocytes into the CNS could be used as a tool to deliver growth factors to the site of CNS injury after brain trauma so as to enhance brain repair.

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