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Migration of Hematogenous Cells Through the Blood-Brain Barrier and the Initiation of CNS Inflammation

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The central nervous system has long been considered an immunologically privileged site. Nevertheless, cells derived from the bone marrow can and do enter the CNS in a number of circumstances. Derivatives of the monocyte/macrophage lineage appear to enter and take up residence in various structures of the CNS as part of normal ontogeny and physiology. Immunocompetent cells, such as T-lymphocytes of both CD4 and CD8 positive groups, enter the nervous system in what appears to be a random fashion when they are activated by antigenic stimulation. These lymphocytes perform the required immunological surveillance of the CNS, and initiate inflammation therein during infectious and autoimmune reactions. In this review, the evidence supporting the above observations is examined, and a hypothesis for the pathogenesis of CNS inflammatory reactions is presented.

Introduction

Among the basic types of pathological reactions occurring in mammals, inflammation is one of the most complex. Our understanding of these processes has expanded rapidly over the past quarter of a century, and now with the increased power of cellular immunology, molecular biology and the availability of transgenic experimental animals, many heretofore obscure processes are being explained.

The Neuropathologist confronts these issues most frequently in association with spontaneously occurring human illnesses. These include the viral encephalitides, brain abscesses, transverse myelitis, and Multiple Sclerosis (MS). When seeking to understand the pathogenesis of any of these conditions, certain key questions arise. One of the most basic of which is: How does inflammation start in an organ that is immunologically privileged? It has long been known that the central nervous system (CNS) of healthy mammals is virtually devoid of leukocytes (1-5). T-cells are virtually undetectable in the CNS parenchyma, macrophage/monocytes are very rare, and even the expression of molecules of the major histocompatibility complex (MHC), which are required to present an antigen to T-cells, is far lower than in any other organ (2,3,6-13). Despite all of these features which would render the CNS an organ immunologically distinct from all others, it is obvious that the immune system can and does locate antigens within the CNS, and appears to have little difficulty is initiating inflammation therein. How?

The blood-brain barrier (BBB) is one issue that must be confronted at an early point in seeking to answer this question (14). It is obvious that any mechanism which physically destroys the components of the BBB will render the CNS open to the cellular and molecular constituents of the blood. This occurs in traumatic or surgical injury, infarction, and hemorrhage. In such circumstances the required participants for inflammation are rapidly delivered to the site of injury in a gross, non-specific fashion. In bacterial meningitis, fungal infections and potentially in brain abscesses, the infectious agent itself produces material which induces incompetence if not frank disruption of the BBB. Here again, the initiation of inflammation most probably proceeds via mechanisms identical to those in any other bodily site, although potentially at a slower and less efficient pace. The most difficult aspect of understanding the beginnings of inflammation in the CNS involves those conditions in which there is no gross nor exogenously induced disruption of the BBB, conditions in which inflammation becomes established prior to and as the cause of opening of the BBB. In the vast majority of illnesses of this type lymphocytes, predominantly T-cells, and monocytes are among the earliest cellular participants. How does such inflammation start in the CNS when the blood-brain barrier is intact?

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There are two principle paradigms for the analysis of the initiation of CNS inflammation. One is the spontaneously occurring human disease, multiple sclerosis. The other is an experimental model with many features resembling multiple sclerosis which is named experimental allergic (autoimmune) encephalomyelitis, or EAE as it is typically called (15-18). In multiple sclerosis the inciting event, the antigen inducing the inflammation, the immunoregulatory controls, and the basic pathogenic insult-be it infectious or truly autoimmune-are all unknown at this time. With EAE, however, a great deal is understood. The antigens capable of causing the illness are myelin basic protein (MBP) (15-22) or myelin proteolipid protein (PLP) (23-26). The cell type causing the illness is a CD4 positive T-lymphocyte (5,27-31). These cells must recognize their antigen not in soluble form, as do antibodies, but bound to a molecule of the MHC on the surface of an antigen presenting cell. Both MS and EAE will produce transient paralysis and focal, perivascular and submeningeal areas of chronic inflammation and demyelination. Although a number of discrepancies exist between EAE in various experimental mammals and MS in humans, there are numerous points at which the two conditions appear to parallel one another (15-18,32).

In order to unveil the basic mechanisms underlying CNS inflammation, the first step is to define which molecular and cellular participants must be present in the CNS (or any other organ) in order to initiate inflammation. At a most basic level, the minimal requirements are: an antigen, an antigen presenting cell, and a T-lymphocyte specific for the antigen. In this review the research findings relative to specific T-lymphocyte mediated experimental models will be discussed and a hypothesis proposed which could explain how inflammation develops across an intact blood-brain barrier. The migration of cells from the blood into the nervous system parenchyma is a central feature.

Antigen Presenting Cells (APC's)

In tissues of the immune system antigen presentation has been shown to be the function of specialized members of the monocyte/macrophage family (33-37). These cells constitutively express the MHC molecules necessary to present small peptides to T-cells in a fashion that is highly immunospecific (38-40). In addition, the APC's elaborate cofactors which are needed to fully stimulate the T-cell to the activated state (36,37,41,42). For immune reactions in the CNS, a central question in neuroimmunology over the past decade has concerned the identity of cells in the CNS which perform this critical function. The primary candidates have been: The astrocyte, the endothelial cell, and the microglia. As with most neuroimmunological investigations, these studies have centered on analysis of multiple sclerosis and EAE. The studies that have been published which

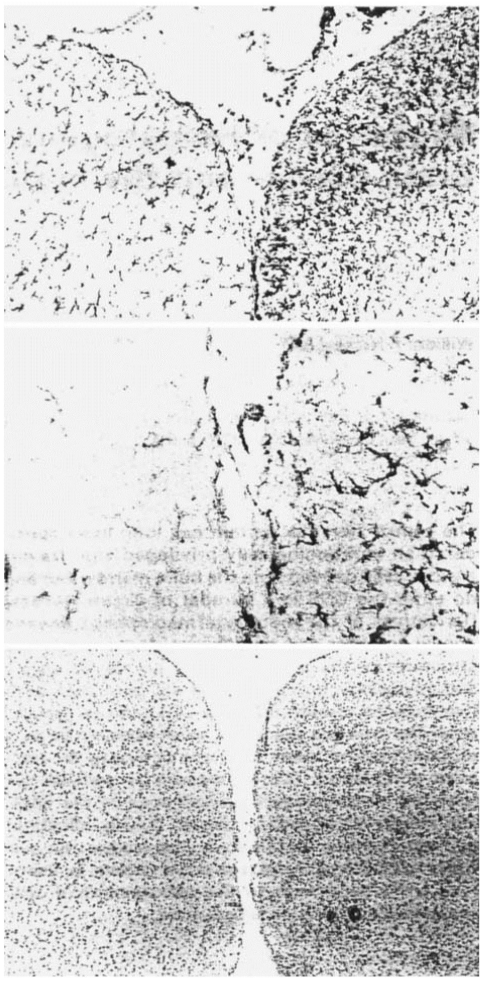

Figure 1 Immunohistochemically stained tissue section of the two superior colliculi of a rat in which one optic nerve had been cut. The stain identifies CD45 (leukocyte common antigen) signifying bone marrow origin of the cells. These cells are microglial cells. The left side demonstrates the microglial proliferation and enhanced CD45 expression induced by nerve transection (x 180). Figure 2 Histological section of the superior colliculi stained for MHC class II antigens. The left colliculus had been deafferented seven days before death by optic nerve transection. The photomicrograph demonstrates the extensive expression of MHC class II molecules needed for antigen presentation by microglial cells. The right side is undamaged, an exhibits only minimal microglial MHC expression (x 230). Figure 3 The photomicrograph shows the superior colliculi of a rat which had the right optic nerve cut 5 days before the development of clinical EAE. The left colliculus, to which the right optic nerve projects, is selectively involved by the perivascular inflammation of EAE, while the undamaged right colliculus is totally spared. (Haematoxylin, x 90).

would support each of the mentioned cell types as the CNS APC are of two main types: immunohistochemical analysis of diseased tissue, and the *in vitro* investigation of the immunological capabilities of isolated/purified cells. Immunohistochemical studies have focused on the detection and cellular distribution of MHC class II molecules in diseased or normal CNS tissue because it is this group of molecules which are necessary for an APC to present an exogenous antigen to a T-cell (33-40). The MHC class II molecules are the "restriction elements" in all defined models of EAE (43,44). *In vitro* experiments have been of numerous types, but have usually centered on the ability of a certain class of cell to elaborate MHC class II molecules on their surface, and to functionally present a defined antigen to T-lymphocytes.

The astrocyte was the first cell to gain attention as the potential CNS APC primarily through the studies of Fontana and collaborators (7,13). They demonstrated that astrocytes could be induced to express MHC class II molecules *in vitro* and also to present MBP to T-cells specific for that antigen (45). Additionally, astrocytes were shown capable of *in vitro* secretion of Interleukin-l, and other important cytokines known to have special roles in immunological responses (46,47).

In multiple sclerosis brains astrocytes were reported as being positive for MHC class II in the area of plaques, and in the CNS of virally infected rats astrocytes were positive for this molecule (48-52). However, a large number of reports examining the CNS of rats with EAE failed to define MHC class II positive astrocytes (12,53,54). One study which did succeed on finding such positive astrocytes noted that they could only be detected in fully established inflammatory lesions in clinically ill rats, and then they were exceedingly rare (3). This was curious since the studies of Fontana, et al. (7,13,45,47) had employed rat astrocytes and lymphocytes, and the presumption would be that rat EAE should be a system in which class II positive astrocytes abound. Thus, the role of the astrocyte as an APC active in initiating inflammation remains unresolved.

The CNS-derived endothelial cell in turn became a focus of study following the immunohistochemical observation its class II positivity in guinea pig EAE (55,56). *In vitro* studies likewise confirmed the ability of rodent endothelial cells to present MBP to T-cell (57-59). Subsequently, in both humans a variety of experimental animals endothelial cell MHC expression has been documented (3,6,8,48,55,56,59). In counterpoint, a number of other investigators have been unable to confirm the MHC class II positivity of endothelial cells in either EAE or MS tissue (4,12, 53,54,60,62). This point is still debated. However, an experiment using rat bone marrow chimeras in which the endothelial cells of the host animal were miss-matched with the antigen presentation requirements of the EAE inducing T-lymphocytes demonstrated that the rats did develop disease (63). This argued strongly that the endothelial cell was not the critical cell for antigen presentation.

The final candidate for the role of CNS APC is the microglial cell. Interest in this obscure cellular constituent has peaked in the past five years. The histogenesis of the microglial cell has long been debated: is it a true glial element or is it derived from the bone marrow? Many studies exist which present data pro and con (64-69). Like the astrocyte and endothelial cell microglia can present antigen to T-cells *in vitro* (70,71). Immunohistochemical studies of humans and experimental animals have documented that MHC class I and II expression can be induced on microglia by a number of methods, and these cells are class II positive in both MS and EAE (6,53,54, 60,62,72-74).

At this time evidence strongly favors the concept that all microglia are derived from the bone marrow. Microglial cells express CD45 (Leukocyte common antigen, Figure 1), a molecule which to date has only been identified on cells of marrow origin (67,73-75); cells and tissues of any other histogenesis do not express this antigen (76). CD45 appears to be a necessary molecule in antigen presentation to T-lymphocytes (77,78). The investigations of Perry and Gordon suggest that microglial cells take up residence in the CNS antenatally (67). In mammals, the microglial cells are disseminated throughout the CNS, and they have recently been reported to constitute part of the blood-brain barrier itself (75).

An immunohistochemical study at both the light microscopic and ultrastructural levels using rat bone marrow chimeras demonstrates conclusively that the normal CNS contains a number of cell types derived from the bone marrow (Hickey, Vass & Lassmann, unpublished data). In these chimeras monocyte related cells were found in the meninges, the choroid plexus around small and large blood vessels, and even in the parenchyma. Although the majority of the marrow derived cells actually in the CNS parenchyma were T-cells, rare cells with the branching morphology of dendritic microglia were detected. The physiological mechanisms which induce these marrow elements to enter the CNS and become permanent residents therein are totally undefined.

Microglial cells have other features which recommend them as the CNS APC. Their cell surface phenotype, determined by monoclonal antibodies, shows them to be close relatives of the monocyte /macrophage/dendritic cell family (61,71-73,79-83). Cells of this lineage function as "classical" APC's in tissues of the immune system in all mammals (33-37).

There are two additional studies which further support the claim of the microglial cell to the role of CNS APC. The first involves the use of Wallerian degeneration which in the rat selectively affects the

contra-lateral superior colliculus following optic nerve transection (84,85). Prior studies have shown that MHC and CD4 molecules are selectively induced on microglial in the deafferented colliculus when the optic nerve is severed (84,85). Following the time course for the appearance of the MHC, CD45, and CD4 molecules on microglia following such an injury, we noted that the MHC class II and CD4 molecules-both required for antigen presentation-begin to be expressed 72 to 96 hours after transection (Figure 2) (Hickey & Molleston, unpublished). In the rat, the superior colliculus is not an anatomic site involved in the inflammation of EAE. However, when the optic nerve is sectioned, the superior colliculus becomes inflamed, and only on the side which has suffered the deafferentation (Figure 3). Interestingly, the susceptibility of the damaged colliculus to EAE is induced within 24 hours of optic nerve transection, early in the phase of microglial reaction. In this experimental setting, susceptibility to inflammation corresponds with the immunological potentiation of microglial cells. The second study involved the use of rat bone marrow chimeras (72,73). In this system rats of strain "A" are lethally irradiated and then given a transfusion of bone marrow from a rat which is the first generation offspring of strain "A" bred with "B", termed (A x B)F1. The resulting rat then has all of its somatic tissues of type "A", but its immune and hematopoetic system is "A x B". If this rat is then given T-lymphocytes derived from strain "B" which recognize MBP, but only when bound to MHC class II of strain "B", these rats develop EAE. Normally "B" lymphocytes have no effect on strain "A" rats. This indicates that something has inherently changed in the constitution of the CNS of the chimera which now renders it susceptible to EAE induced by strain "B" T-cells. An APC from the transfused bone marrow has entered the CNS, and is now functioning there. What is this cell?

In the classic work of Hortega (64), a cell identified as "perivascular microglia" were defined. These constituent cells are "closely applied to the walls of vessels, resting on the outer surface of the adventitia...these microgliocytes in contact with the vessel are multipolar elements that either run across the vessel or are parallel to the latter". It is this cell in the CNS parenchyma which has repopulated from the bone marrow in the rat chimeras that is now functioning as the APC (72,73). Immunohistochemical and ultrastructural studies of this special cell reveal that it has a cell surface phenotype identifying it as a member of the monocyte family. These cells are part of the normal make up of the rodent (72,75,79,80) and human (8) CNS.

Thus we return to a central, persistent issue in neuroimmunology. What are the cellular or molecular signals which cause the CNS during development to become populated with the precursors of the parenchymal microglia? What continues through

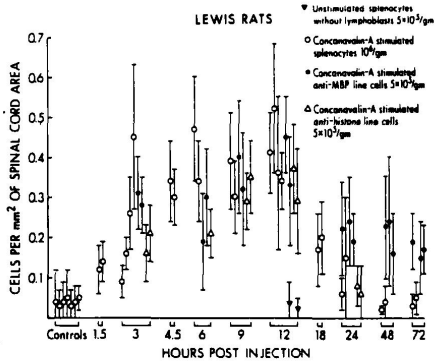

Figure 4 This graphically demonstrates the time related appearance of T-cells in the rat CNS following intravenous injection. Each bar represents the mean ± the SD for the concentration of cells found in 1.0 cm2 of spinal cord parenchyma in one rat. Control animals received saline injection without cells.

post natal life to attract cells of the monocyte/dendritic cell group to continuously repopulate the CNS with perivascular "microglia"? Both of these events are part of normal development and physiology; however, the exact controls governing their migration to the CNS is unresolved. Discovering the answer to these questions will undoubtedly extend our understanding not only of neuroimmunological phenomena, but also may provide insight into the spread of HIV to the CNS and may offer a way to direct genetically altered cells into the CNS without direct implantation.

T-Lymphocyte Entry into the CNS

The above discussion demonstrates that APC's needed for the initiation of inflammation enter the nervous system from the circulation. How do T-cells, one other additional requirement for inflammation, arrive? Here again, the EAE model offers some clues. Over the past decade it has been reported that EAE can be transferred to a recipient rodent only if the cells are in an activated form. T-cells stimulated to the blast phase, either by their specific antigen (86-89) or by non-specific, mitogenic lectins (87-90), will produce EAE. Unstimulated cells will not (86-89).

In recent years great interest has developed in immunology concerning "homing receptors", molecules on the surface of cells which specifically target them to certain tissues, and "molecular addressins", endothelial cell surface molecules which interact with the homing receptors to inform the hematogenous cell that a specific site is appropriate for egress (91-93). Lymphocyte "homing" entails the concept that these cells will circulate until there

occurs a specific interaction between the lymphocyte surface and a vascular bed in a particular tissue. A corollary of this would be that certain types of lymphocytes are predestined to enter certain organs. To date there have been defined neither homing receptors nor molecular addressins which are specific for the nervous system. It is well documented that in experimental animals and man T-cells are very rare in the CNS parenchyma (1-5,61,94), yet they possess some inherent ability to enter and initiate inflammation.

Multiple reports have characterized the T-cell receptor genes used by lymphocytes to recognize MBP, the antigen causing EAE and implicated in MS (5,16,19-21,95). There is a striking uniformity of selection of the type of T-cell receptor that is used to recognize this antigen in rodents (95-99). This discovery provides a potential molecular basis for the hypothesis that only cells specific for a CNS antigen will enter the nervous system (99). Cross and colleagues (100) have examined the apparently specific homing of such encephalitogenic cells to the CNS in mice. Yet, there are observations that argue against the contention that lymphocytes enter the CNS by an antigen-specific homing mechanism.

Meyermann and co-workers (101,102) documented the fact that T-lymphocytes specific for ovalbumin-not a normal constituent of the rat CNS-enter that organ as readily as cells specific for MBP. This led Wekerle, Linnington, Lassmann and Meyermann to set forth the hypothesis that any T-cell which has been stimulated to the blast phase can readily penetrate the blood-brain barrier (102). To investigate the constraints on this system, an extensive study was performed to dissect the immunological features of T-cells that govern their entry into the CNS (73,103, 104). Rats were injected intravenously with one of four different types of cells. The first type consisted of spleen and lymph node cells which had been grown *in vitro* in such a way as to discourage lymphoblast transformation. The few blasts present in this cell pool were removed and the unstimulated T-cells (60% to 70% of the pooled cells by flow cytometry) were injected. The second type of cells were 85% T-lymphoblasts which had not been selected for any single antigenic specificity, hence they would represent a random sampling of the rat's T-cell repertory. This group could not be claimed to be specific for CNS antigens. The third and fourth types of cells were both 95% T-lymphoblasts, and both were highly specific for a single distinct antigen. The third type of cells recognized the encephalitogenic antigen MBP; rats injected with T-lymphoblasts of this type would develop EAE within 96 hours. The fourth type of T-cells recognized calf thymus histone, an "irrelevant" antigen with the same physicolchemical characteristics as MBP; however, T-cells specific for this antigen produce no illness in experimental animals. These last three types were cells that had been stimulated to the lymphoblast stage by the mitogenic lectin concanavalin-A.

The results of this investigation are shown in Figure 4. The above experiments were repeated by injecting the same types of Lewis rat T-cells into the circulation of MHC allogeneic rats. In this setting it was impossible for the T-cells to recognize a CNS specific antigen on the CNS endothelium because of the mismatch in MHC antigen presenting molecules (72,73). The results were virtually identical. The extended study revealed that T-cell entry into the intact CNS was independent of antigen specificity (an hence, probably independent of T-cell receptor gene usage), tissue compatibility with the host, and the phenotype of the T-cell (CD4 vs. CD8 positive). Indeed, entry appeared to be only linked to the activation state of the T-cell; lymphoblasts entered, unstimulated cells did not. One very interesting aspect of this study is the rapidity with which T-lymphoblasts entered the central nervous system. Following injection into the rat's circulation detectable, newly arrived T-cells could be found in the spinal cord within 90 minutes. Their peak concentration occurred between nine and twelve hours following injection.

The T-cell concentration declined after twelve hours returning to baseline levels in one to two days. However, if the cells which seemed to enter the CNS in a random fashion were specific for an antigen in the CNS, such as MBP, their concentration remained elevated above baseline for days (Figure 4). This suggests that activated T-cells, specific for any antigen, will survey the CNS when they circulate. If they find their antigen, they will stop and initiate inflammation, providing their concentration reaches a sufficient threshold.

One additional finding of this study was that tissue penetration by T-lymphoblasts appeared to be random since the T-lymphoblasts were found to distribute to virtually every organ in the body following injection. A recent study in sheep has reached similar conclusions and demonstrated that normal circulating, unstimulated T-cells have a vastly different tissue distribution pattern than do T-cells which have been previously stimulated by antigen (105). While these studies do not necessarily contradict the concept of specific T-cell homing to the CNS, they do suggest that the dissemination of T-lymphoblasts may be governed by different rules other than those operating in classical homing.

The nature of the changes occurring in T-lymphocytes when they enter the blast phase which empower them to enter the CNS remain to be defined. The available data suggests that the controlling features of the system reside in the T-cell, not in the CNS endothelial cell. If T-cells in blast phase can readily enter the CNS within a few hours of injection, the interval is too short for de novo synthesis and expression of endothelial addressins. There was no

stimulus given to the rats to induce enhanced expression of migration related molecules on the endothelium. Moreover, the apparently random distribution of injected T-lymphoblasts through all the bodily organs examined, regardless of the T-cells antigenic specificity, argues that the activated T-cell is recognizing some ligand which is constitutively expressed by endothelial cells of most vascular beds. The T-lymphoblast must express on its cell surface a migration related molecule not exhibited by resting T-cells. It has been documented that activated T-cells exhibit enhanced adhesiveness through increased expression of LFA-1 following activation (106). Also, novel synthesis of heparin endoglycosidase needed to degrade basement membranes occurs following T-cell activation (107), furthermore competitive inhibitors of this enzyme prevent the development of EAE in rats (108). Finally, investigations in this laboratory have identified the expression of novel cell surface antigens that appear on rat T-lymphoblasts following activation (Hickey & Palmer, unpublished observations); however the molecular nature of these changes and their potential relationship to cell migration are incompletely characterized.

Other questions which remain concerning the entry and persistence of T-cells in the CNS are: Do cells which pass through the CNS return once again? How long can lymphoblasts remain in the circulation before entering a tissue? Once a T-cell specific for a CNS antigen enters the CNS can it be cyclically reactivated in the CNS, or must it return to the organs of the immune system for reactivation?

Initiation of CNS Inflammation: A Hypothesis

Considering the above information it is possible to construct a hypothesis describing the pathogenetic mechanism for the initiation of inflammation across an intact blood-brain barrier. In this hypothesis, the transgression of the blood-brain barrier by cells of the lymphocyte and monocytic classes while leaving that barrier intact, is a central, albeit poorly understood, feature. The normal CNS contains hematogenously derived elements that enter the CNS as part of the normal mammalian physiology. The parenchymal microglia enter during development, their meningeal and perivascular relatives enter continuously during life. The perivascular cells, and potentially the parenchymal microglia as well, are ever in a state of readiness to function as antigen presenting cells.

T-lymphocytes are activated in the lymph nodes and other tissues of the immune system. These lymphoblasts then enter the circulation in search of their specific antigen. They randomly distribute throughout all the organs of the body, including the CNS. If they fail to detect antigen(s), they pass through the tissue leaving it morphologically undisturbed. If they encounter their antigen, in the context of the appropriate MHC molecule, on the surface of an antigen presenting cell, a certain percentage of the cells will persist in that organ and initiate the process of inflammation. Once this process is started, the blood-brain barrier is opened, molecular signals to attract other lymphocytes and monocytes are elaborated, and other cells from the circulation plus immunoglobulins, coagulation and complement factors enter. All the needed participants for a typical immunologically mediated inflammatory reaction thereby arrive in the nervous system. Inflammation as we define it has begun.

Under this hypothesis, any foreign antigen present in the CNS to which the immune system has not been exposed would be tolerated. This phenomenon has been documented by the implantation into the CNS of foreign tissue grafts which would be rapidly rejected if placed elsewhere in the body normally exposed to the immune system's elements (109). Such grafts are tolerated and survive without immunological damage. However, when such engrafted animals are challenged in the periphery with the same antigen, and the immune system in the normal manner comes to recognize it as foreign, then the heretofore tolerated CNS graft is rapidly located and destroyed (109). The T-cells which have always been in the host's immunological repertory, are now stimulated and empowered to enter the CNS and survey it for that antigen.

According to this hypothesis, any activated T-cell would have the ability to pass the blood-brain barrier and "survey" the CNS for its antigen. This is in keeping with the observation that even healthy adults do have within their CNS and cerebrospinal fluid low but detectable numbers of T-cells. These few cells would be a reflection of the small numbers of activated T-cells continuously in the circulation in response to the low but continuous stimulation from environmental pathogens every individual confronts. Thus, the brain and spinal cord benefit from the protection of the immune system, but are not subject to continuous passage of normal circulating lymphocytes which have yet to be activated.

Finally we return to the human neurological illnesses characterized by inflammation. The frequently observed vasocentricity of lymphocytic aggregates in viral encephalitis and multiple sclerosis can be explained since it is in the perivascular area that the critical antigen presenting events are occurring.

The above scheme is only a hypothesis, but it is in keeping with most of the observed phenomena concerning CNS inflammatory conditions. Further studies will refine the various aspects of the early events in CNS inflammation which may support or disrupt the hypothesis. Nevertheless, it is certain that a more complete understanding of the mechanisms by which APC's are attracted to the nervous system, and the changes occurring in T-cells permitting them to survey the CNS, will greatly enable us to deal effectively with the pathological effects of human neurological inflammatory diseases.

Acknowledgments

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