The Role of T-Cell-Mediated Mechanisms in Virus Infections of the Nervous System

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1 Introduction

For quite a long time, the paucity of immune system elements in the central nervous system (CNS) and its tight enclosure by the blood–brain barrier (BBB) have led to the assumption that this organ is an immunologically privileged site of the body that is rigorously excluded from immune surveillance. However, data accumulated in recent years suggest that this view has to be revised substantially, since the healthy as well as the injured CNS performs an intense and permanent cross-talk

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G. Gosztonyi (ed.), *The Mechanisms of Neuronal Damage in Virus Infections of the Nervous System*

with the immune system resulting usually in a well tuned and gradually adapted immune response in the brain (CSERR and KNOPF 1992; FABRY et al. 1994).

The healthy non-affected CNS is indeed an immunologically quiet organ with very little traffic of lymphoid cells and, dependent on the species, with no or very basal expression of major histocompatibility (MHC) antigens. Due to its vulnerability and poor capability of regeneration and in view of potentially tissue damaging properties of an immune response, it is not surprising that the CNS actively maintains this state of low immunoreactivity by creating a hostile environment for inflammation. This is mainly achieved by CNS-localized synthesis and maintenance of high levels of immunosuppressive substances for pro-inflammatory T lymphocytes, e.g. transforming growth factor (TGF)-β or nerve growth factor (NGF) (CSERR and KNOPF 1992; NEUMANN and WEKERLE 1998). Therefore, the BBB not only protects the CNS from challenges coming from the periphery of the body, but is also needed to keep the level of immunomodulatory substances high and prevent drainage of these important regulators into the periphery.

In case of injury or invasion of infectious agents, the state of immunological silence must be overcome very rapidly in order to efficiently defend the integrity of the organ. Virus infections of the CNS demonstrate very clearly the conflict which has to be solved by the immune system. The ultimate goal is a rapid and complete elimination of the virus. Nevertheless, in the attempts to achieve this goal, extreme care must be taken to keep tissue damage and functional disturbances on the level of neurons as low as possible. In this context, effector functions of $CD8^+$ T lymphocytes are of particular interest, since they can cause death of virus-infected cells by induction of the apoptotic pathway, either through delivery of enzymes through pores formed in the membrane of the target cell or by interaction with the Fas (CD95) molecule on the virus-infected cell (FROELICH et al. 1998). It is obvious that these potentially dangerous properties of $CD8^+$ T lymphocytes require strict control.

Cellular elements that exert regulator functions in the course of intracerebral immune reactions are provided by both the immune and the central nervous systems. It is the $CD4^+$ T lymphocyte which can overcome the immunosuppressive milieu of the CNS by secretion of pro-inflammatory tumor necrosis factor (TNF)-α and interferon (IFN)-γ. The latter substance is a potent inducer of MHC antigens on CNS resident cells like microglia, which thereby rapidly acquire antigen-presenting capabilities for both $CD8^+$ and $CD4^+$ T lymphocytes. Intimate contacts between $CD4^+$ T lymphocytes and antigen-presenting microglia are of major importance for attracting inflammatory cells from the blood, for homing of effector leukocytes to virus-infected areas and probably also for down-regulation of immune effector functions as well as for induction of tissue-damaging properties in microglial cells. This concert of regulation is further complicated by close interrelationships between activated microglia and other cellular elements of the CNS, mainly astrocytes and to a certain extent nerve cells, which in turn might act back on the tissue-infiltrating T lymphocyte population.

This review will focus on some recent aspects of the role of the T lymphocyte compartment in the course and the outcome of viral CNS infection. Due to the

extreme difficulties in obtaining clinical tissue specimens from the brains of patients suffering from acute viral CNS infection, almost all data about the immunological events and their dynamics in the brain parenchyma have been collected in natural and experimental animal models of virus-induced CNS disease. Although these models might not exactly describe the situation in humans, they have proven very valuable in our understanding of how the intracerebral immune system response is organized and how it determines clinical course and outcome of viral CNS infections to an extent which is much larger than we thought a few years ago.

2 Expression of Immunoregulatory Molecules in CNS Tissue

As pointed out in the Introduction, in the unaffected CNS constitutive expression of molecules which have regulatory functions in the immune system response is very rare. Nevertheless, coordinated action of T lymphocytes in the virus-infected brain tissue depends entirely on the presence and correct regulation of expression of these molecules. Therefore, in this section a brief discussion will focus on expression of the most important molecules which govern T cell activity.

2.1 Major Histocompatibility Antigens

Considering that antigen recognition of T cells depends entirely on presentation of antigenic peptides in the groove of MHC molecules, expression of these structures must be of critical importance for both $CD4^+$ and $CD8^+$ T cells in the virus-infected brain. In contrast to many other organs of the body, it was believed until recently that the CNS is part of a group of immunologically silent sites like the eye, the synovial spaces of the joints and the testis, which in a healthy state do not express MHC molecules. At least for the CNS, the absence of MHC antigens is not absolute. There are differences between species and even within one species there might be differential expression of these molecules in inbred strains of animals. For instance, Brown Norway rats express class II MHC antigens constitutively in healthy brain tissue on a level which is easily detectable by conventional immunohistochemistry. In contrast, Lewis rats show no or an extremely weak expression of these molecules in situ (Sedgwick et al. 1993). These data indicate that under certain, so far unknown, circumstances, the unaffected brain is by no means immunologically secluded but is in a state of immunological alertness that allows immediate interaction with α/β T cell antigen receptor (TCR)-expressing T lymphocytes penetrating into the tissue.

Independently from the level of MHC antigen expression in the healthy brain, both class I and class II antigens are up-regulated strongly in the course of viral CNS infection. This poses a very important question, which to date is discussed very controversially, namely: What is(are) the cell type(s) which express and

up-regulate MHC antigens in the brain? There is no doubt about the fact that infiltrating lymphoid cells are MHC-positive. This is true for all cells with respect to MHC class I and for many cells with respect to MHC class II. Inflammatory monocytes/macrophages, some T cell subsets and B lymphocytes do express class II antigens. However, whereas the latter expression serves mainly inter-lymphoid communication, it would be of major interest to know what type of brain-resident cell expresses MHC, because this will decide and influence in many ways the handling of T lymphocytes in the tissue.

2.1.1 Class I Major Histocompatibility Antigens

It is generally accepted that MHC class I expression can be up-regulated on microglia, astroglia and oligodendroglia by IFN-γ (Wong et al. 1984). This would allow interaction of $CD8^+$ T cells with these brain-resident cells in an antigen-dependent manner, either in order to destroy the contacted cell or to receive regulatory signals. In contrast there is considerable controversial discussion about the capability of nerve cells to express class I antigens. The answer to this question is of tremendous importance, because many viruses can infect nerve cells. In the absence of MHC class I expression, virus-infected nerve cells would be unrecognized by $CD8^+$ cytotoxic T cells, and up-regulation of these molecules would lead to a high risk of irreversible destruction of infected nerve cells resulting in a lethal threat to the host. Conflicting data were reported on this issue. Early data from Joly et al. (1991) demonstrated very clearly that retroviral-transformed neurons can transcribe the genetic information of the MHC class I heavy chain, albeit on a level which is only 1%–3% of that detected in a fibroblast. In contrast, the other structural element of a functional MHC class I molecule, β2-microglobulin, is expressed at a level which is comparable to that found in fibroblasts of the same mouse strain. Thus, neurons are unable to express sufficient intact MHC class I molecules that would allow their killing after virus infection. In vivo data from the same laboratory confirmed this assumption (Mucke and Oldstone 1992). During persistent infection of mice with lymphocytic choriomeningitis virus (LCMV), the virus is harbored in nerve cells, whereas during acute infection the virus is detected in cells of the leptomeninges. Transfer of $CD8^+$ T lymphocytes with specificity for LCMV causes very severe neurological disease in acute infection but not in persistent infection. Histopathological studies revealed that in the latter case no MHC class I expression could be observed, although neurons are heavily infected by the virus. In vitro, LCMV infection of neurons also fails to up-regulate MHC class I molecules, whereas stimulation of these cells with IFN-γ causes up-regulation of class I antigens and renders these cells susceptible to $CD8^+$ T cell-mediated killing.

These data are in sharp contrast to work from other investigators (Bilzer and Stitz 1994), who reported strong up-regulation of MHC class I antigens on neurons of rats which were infected by Borna disease virus (BDV). Since neurons in the hippocampal area are the main target of this virus, one would expect that strong $CD8^+$ T cell infiltration of this area would cause extensive damage. Indeed it has been shown by Bilzer and Stitz (1994) and others that $CD8^+$ T cells are an

important pathogenic factor in BDV-induced CNS disease. A straightforward and simple explanation for these discrepancies could be the fact that virus-infected neurons are often wrapped by very fine and thin microglia processes. Up-regulation of MHC class I molecules on the microglia could suggest expression of these molecules on neurons if, as was done by Bilzer et al., the tissue is examined at the light microscopic level. Immune electron microscopy should solve this problem. Moreover, published work by NEUMANN et al. (1995) adds substantial support to the idea that expression of MHC class I on neurons is subject to very tight intracellular and extracellular control mechanisms. Analysis of neurons on a single-cell basis by patch-clamp techniques, confocal laser microscopy and reverse transcription (RT)-polymerase chain reaction (PCR) disclosed the remarkable fact that transcription of MHC class I heavy chain was rare in neurons with spontaneous electrical activity, whereas in electrically silent neurons both MHC class I heavy chain and β2-microglobulin were transcribed. However, β2-microglobulin transcription was more tightly controlled than that of MHC class I mRNA. Despite transcriptional activity no surface expression of MHC class I molecules was detectable in these "electrically defective" neurons. Treatment of these cells with IFN-γ resulted in detectable amounts of MHC class I at the cell membrane, making these cells susceptible to killing by virus-specific T cells. TNF-α, a potent stimulator of MHC antigens, acts differently on damaged neurons than does IFN-γ. Although strong up-regulation of MHC class I heavy chain transcription is detectable, a comparable effect on the β2-microglobulin message is absent, and consequently no expression of class I molecules is detected on the cell surface (NEUMANN et al. 1997).

A very careful conclusion from these data would be that nerve cells can transcribe the message for the heavy chain of MHC class I; expression of the molecule, however, is limited to severely injured neurons that are exposed to IFN-γ.

2.1.2 Class II Major Histocompatibility Antigens

In contrast to MHC class I antigens, expression of MHC class II antigens in the brain is less controversial. There is no doubt that brain endothelial cells, pericytes, microglial and astroglial cells can express these important regulatory molecules and that IFN-γ, and to a certain extent also TNF-α, are the important mediators of induction (FIERZ et al. 1985; FONTANA et al. 1984; FREI et al. 1987; MALE et al. 1987; PARDRIDGE et al. 1989; SEDGWICK et al. 1993; SEDGWICK et al. 1991b). In vivo, these mediators are very often supplied by infiltrating T lymphocytes – a fact that is reflected by observations that, in the course of virus-induced damage of the CNS, the dynamics of MHC class II expression on microglial cells follow the influx and disappearance of $CD4^+$ T lymphocytes in the tissue. Nevertheless, care has to be taken when looking at in vitro and in situ expression of these molecules in virus-infected cells. In astrocytes, MHC class II expression can be induced in vitro by IFN-γ (FIERZ et al. 1985). Very interestingly, viruses themselves, even if non-infectious, can up-regulate MHC class II on astrocytes in vitro in the presence of IFN-γ-neutralizing antibodies. This was shown by our laboratory earlier for the coronavirus JHM in primary rat astrocytes (MASSA et al. 1986). However, in situ,

expression of MHC class II on astrocytes is difficult to observe even under conditions of severe inflammation (Pope et al. 1998; Tontsch and Rott 1993). Today it is known that MHC expression on astrocytes is tightly controlled by neurons (Neumann et al. 1996; Tontsch and Rott 1993). Hippocampal slice cultures from rats revealed only moderate class II up-regulation after treatment with IFN-γ and this up-regulation was focused on areas of severe neuronal degeneration. The same observation holds true for microglial cells; however, inducibility of class II antigens is less tightly controlled than in astrocytes. After poisoning neurons in the tissue slice with a sodium channel blocker and subsequent treatment with IFN-γ, class II molecules were up-regulated on both astrocytes and microglial cells, also in areas of intact neuronal architecture. Nevertheless the level of MHC class II expression on microglial cells was much higher than in astrocytes. It seems that intact neurons actively control the expression in their surroundings of MHC antigens that are important in immunoregulation, but in contrast to microglia, in astrocytes this control is much more stringent (Neumann et al. 1996). These data indicate that microglia are much more alert with respect to inducible antigen-presenting cell (APC) function, an interpretation which is supported by in vitro data showing easy induction of antigen-presentation in microglia, whereas astrocytes are more restrictive in providing these functions (Sedgwick et al. 1991a). Possible mediators in this control function are neurotrophins released by intact neurons. NGF, brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) all actively suppress class II inducibility on microglial cells, whereas blockade of neuronal function by toxins or glutamate antagonists restores IFN-γ mediated inducibility of class II antigens (Neumann et al. 1998).

2.2 B7-Molecules

The fact that microglial cells (perivascular and ramified cells) can be induced by T lymphocyte-derived IFN-γ to up-regulate MHC class II expression has fed the speculation that priming and activation of T cells during virus infection of the brain might also occur in the CNS. In this case more than MHC molecules have to be expressed on microglial cells, namely, B7-molecules, which are necessary as a ligand for CD28 on T lymphocytes. Interaction of B7.2 with CD28 provides the second signal which is necessary, beside MHC/TCR engagement, for activation of unprimed T cells (June et al. 1994).

Very recent work from the Theiler's virus model of demyelinating encephalomyelitis unraveled interesting aspects of differential expression of B7.1 and B7.2 molecules in the virus-infected brain (Pope et al. 1998). In contrast to inflammatory T cells, which express predominantly B7.2, nearly all MHC class II-positive macrophages and microglia were also positive for both B7.1 and B7.2. This is of particular interest because interaction of B7.1 with its counterpart CTLA-4 (CD 152) on the proliferating activated T lymphocyte can cause apoptosis (Scheipers and Reiser 1998). Therefore, the brain-resident microglial cell must be considered as a potent regulator of T lymphocyte activity.

2.3 Fas (CD95)/FasL

Expression of the CD95 molecule renders a cell susceptible to induction of the apoptotic pathway, induced by interaction with the appropriate ligand, FasL. This regulatory event is an entirely physiological mechanism which is used to control cell proliferation during ontogenesis and in the course of specific immune reactions. However, the signal transduced by CD95/FasL interaction does not necessarily result in apoptosis (LYNCH et al. 1995). Dependent on the state of differentiation, engagement of CD95 on prelytic $CD8^+$ T lymphocytes by $CD4^+$ T-lymphocyte-expressed FasL during MHC-mediated contact with APC can trigger differentiation of $CD8^+$ T cells to effector killing cells in an interleukin (IL)-2-independent manner. As such it may play a role in viral CNS infections during expansion of antigen-specific $CD8^+$ T lymphocytes in peripheral lymphoid tissue. Besides control of lymphoid expansion and elimination in immune system responses, killing of virus-infected target cells by class I-restricted $CD8^+$ T lymphocytes can also be mediated by this effector mechanism.

In search for CD95/FasL expression in the body, it turned out that some so-called immunologically sequestered organs, like joints, testis and eyes, express FasL at a high density (GRIFFITH and FERGUSON 1997). At present, this is interpreted as an immunosuppressive action of these organs against inflammatory T cells, which all express CD95 when activated. Although not expressed constitutively in the CNS, CD95 and FasL can be up-regulated on brain-resident as well as infiltrating lymphoid cells during inflammation of the CNS, as in autoimmune encephalomyelitis or virus infection (BECHER et al. 1998; CHOE et al. 1998).

3 Afferent Events

3.1 Signaling from the CNS to Peripheral Lymphoid Tissue

Due to the absence of a regular lymphoid drainage system and the lack of dendritic leukocytes in the brain, it is difficult to understand how and where T lymphocytes come into contact with antigens produced in the course of viral CNS infection. As has been shown very elegantly by Cserr and coworkers, priming of the immune system- vs brain-derived antigens occurs in peripheral lymphoid tissue. Radioactively labeled antigens that are applied to the ventricular cerebrospinal fluid (CSF) without damage to the BBB appear very rapidly in the superficial and deep cervical lymph nodes (CLNs), and a strong antigen-specific antibody response consisting mainly of IgM is noted within a few days (CSERR et al. 1992a). In contrast, the contribution of the spleen to this primary response is only marginal. Most likely, the applied antigen is drained with CSF which constantly leaks at the sealing points, which are formed by the meninges and the sensory brain nerves. One of the major places of antigen drainage is the cribriform plate, where the olfactory

nerves penetrate the base of the skull. Antigen leaving the CNS at this site is transported by the oral mucosa to the lymphoid tissue of the neck area (Cserr et al. 1992b).

In the case of experimental viral CNS infection, this drainage system allows priming of the naive peripheral immune system. Natural viral infection of the CNS, however, occurs only under very rare circumstances directly via entry of virus particles into peripheral nerves and retrograde transport. Generally, such infections are the consequence of a concomitant or preceding infection in peripheral organs of the patient. Therefore, virus-specific priming of the T cell compartment occurs in secondary lymphoid tissues which are in close proximity to the viral entry site in the body, and contact of CNS-derived viral antigen in the cervical lymph nodes often represents a secondary encounter of already primed T cells with the antigen.

3.2 Activation and Differentiation of T Cells

Since CLNs are one of the major sites where T cells come into contact with brain-derived antigens, then activation and differentiation into effector cells should occur at this site. As we noted, antigen-specific, proliferative T cell responses are detectable in CLNs of experimentally infected animals a few days after intracerebral application of the virus (Imrich et al. 1994). Interestingly, in rodent models of viral encephalitis, quality as well as quantity of the CLN-located T cell response differs between inbred strains. The intracerebral infection of Lewis and Brown Norway rats can serve as a vicarious example to demonstrate the role of the genetic background in the immunological handling of a viral CNS infection. Both rat strains are susceptible to intracerebral infection with the murine hepatitis virus (coronavirus) strain JHM (JHMV); however, whereas in Lewis rats a paralytic course of the infection may cause death in a third of all animals within a week, Brown Norway rats never show any clinical signs of infection (Watanabe et al. 1987). It is noteworthy that Lewis but not Brown Norway rats develop an autoimmune reaction against basic myelin protein (BMP) (Watanabe et al. 1983), which is a cell component of the viral target cell in the CNS, namely, the oligodendrocyte. The dynamics of the proliferative T cell response in the CLNs of these animals disclose remarkable differences. In 60%–70% of Lewis rats which survive the infection and recover from neurological disease, CLN-derived T cells exhibit ex vivo proliferation without further antigenic challenge in vitro (Imrich et al. 1994). Proliferating activity of these T cells starts immediately after intracerebral infection and increases up to 12 days post-infection, when severity of the neurological disease peaks and the animals start to recover. Proliferation cannot be substantially enhanced by addition of viral antigen into the cultures. Obviously, intracerebral infection of this rat strain induces a strong and polyclonal activation of T cells in the CLNs, albeit with a considerable delay after onset of neurological disease. In sharp contrast, CLN-derived T cells from JHMV-infected Brown Norway rats show extremely low ex vivo proliferation for many weeks after infection, but a robust proliferative response to in vitro stimulation with viral

antigens (IMRICH et al. 1994). Most remarkable, this virus-specific T cell response in the CLNs is biphasic, with a very early response within a few days after infection and a second wave approximately 2 weeks post-infection. In this rat strain, activation of the T cell compartment is rapid, restricted to a few clones and can occur multiple times in the course of the infection. Of course it is tentative to speculate that differences in the CLN-localized T cell reactivity of Lewis and Brown Norway rats mirrors in some way the completely different clinical and histopathological course of the infection. As will be discussed later in this review, it is indeed conceivable that quality and quantity of the peripheral T cell response in this animal model has a causative link to the way that these animals restrict viral spread in the brain and thereby minimize tissue destruction at the very beginning of the infection.

4 Efferent Events

4.1 Extravasation of T Cells

After clonal expansion and differentiation in the periphery, effector T cells must transmigrate through the BBB formed by the wall of brain blood vessels and adjacent foot processes of astrocytes. In this context, a whole series of questions arises: (1) What is the nature of the transmigrating T cell? (2) Is antigen specificity of T cells of importance for extravasation? (3) Do brain-resident cells influence the endothelial layer in a way that might help T cells to transmigrate through the BBB? (4) What signals are given to T cells that have migrated to the perivascular space and the brain parenchyma, and what are the consequences of this signaling? Only few, if any of these questions can be answered in detail for virus-specific T lymphocytes.

Both studies on lymphocyte traffic in sheep and an experimental autoimmune disease of the brain, experimental allergic encephalitis (EAE), in rats have paved the way for studies in this difficult field of CNS-localized immunoreactivity. Today, it is fairly well accepted that freshly activated and non-activated but primed memory T cells can enter any non-lymphoid tissue on a random basis independent of their antigenic specificity (WILLIAMS and HICKEY 1995). As pointed out above, in most cases of viral CNS infection the invading agent entered the host somewhere in the periphery, which then provoked a primary T cell response in the regional lymphatic tissues. Therefore, T lymphocytes with virus-specificity are already recirculating in the vast pool of peripheral T lymphocytes during initial replication of the virus in the CNS. What causes adhesion and extravasation of T cells at the site of infection?

Pathological events associated with viral replication in the CNS most likely cause initial up-regulation of adhesion molecules on the endothelial cell. Viruses are able to infect brain endothelial cells (BRANKIN et al. 1995; COSBY and BRANKIN 1995; CZUB et al. 1995; GAIRIN et al. 1991; GEORGSSON 1994; KRAKOWKA et al. 1987), and it is known that this event can not only up-regulate MHC antigens

(Gairin et al. 1991) but also molecules such as intercellular adhesion molecule (ICAM)-1 (Brankin et al. 1995), which are important for transendothelial migration of T lymphocytes (Greenwood et al. 1995). Besides increased stickiness of T cells at infected BBB endothelium, expression of MHC class I loaded with viral peptides probably causes $CD8^+$ T lymphocyte-mediated destruction of these targets – as has been suggested in LCMV infection of rodents (Doherty et al. 1990). This would result in serious damage of the BBB, creating further activation of the surrounding endothelial layer.

Moreover, although not understood in detail signals are transmitted from inside the CNS to the BBB, which cause luminal up-regulation of adhesion molecules on the endothelial cells. Infection of the brain by retroviruses such as human-, simian- and feline immunodeficiency virus (HIV, SIV, FIV) activates brain-resident microglia cells to secrete IL-1 and TNF-α (Nottet and Gendelman 1995), two cytokines which are known to stimulate expression of endothelial adhesion molecules, e.g. vascular cellular adhesion molecule (VCAM)-1 (Wekerle et al. 1991). VCAM-1 expression on brain endothelial cells seems to be important for adhesion of T lymphocytes in viral CNS infections (Christensen et al. 1995; Marker 1995; Soilu Hanninen et al. 1997) and in CNS inflammatory conditions (Weller et al. 1996).

Additional interesting observations come from model systems of tissue grafting to the brain (Ishihara et al. 1993). Iso- or autografting of skeletal muscle cells onto the surface of the brain (e.g. implantation into the fourth ventricle, between medulla oblongata and cerebellum) provides a focal opening of the BBB, which in other places is otherwise undisturbed. Whereas solutes enter the brain directly through the vessels of the graft, macrophages penetrate into the CNS through vessels that are adjacent to the graft rather than through vessels in the graft. The reason for this is the up-regulation of ICAM-1 on endothelial cells in the medulla close to the grafting site. This experiment demonstrates that a focal pathological event in the brain can result in activation of adjacent endothelial cells which will cause adhesion of activated mononuclear cells.

Once activated T lymphocytes have attached to an endothelial cell, they will enhance expression of adhesion molecules (ICAM-1) by secretion of IFN-γ and TNF-α (Marker et al. 1995), thereby facilitating the adherence of more T cells (Del Pozo et al. 1996). Adherence of cells is then followed by transmigration into the perivascular space. Chemokines and adhesion molecules like T cell-expressed lymphocyte functional antigen (LFA)-1 and endothelial ICAM-1 are deeply involved in the cascade of events triggering this process (for a detailed discussion see Springer 1994).

4.2 Homing of T Cells to Virus-Infected Sites

Once an activated T lymphocyte has passed the BBB, its fate depends on two aspects: (1) Is there antigen presentation in the perivascular space? (2) Is the TCR specific for any of the presented peptides? As has been shown by Hickey et al.

(1991), the amount of activated T cells in the perivascular space drops rapidly to undetectable levels if no antigen-specific contact is possible with perivascular macrophages. T cells that are specific for a tissue-localized antigen are detectable much longer in the perivascular space. These cells interact with antigen-presenting macrophages, a process which leads to mutual stimulating events between incoming T cells and local APC. At the port of entry the APC are perivascular microglia (HICKEY and KIMURA 1988).

The regulating events that guide T cells through the tissue to the virus-infected area of the brain are completely unknown. Although purely speculative (LAMPSON 1998), it seems likely that, immediately after penetration of T cells into the brain tissue, the direction of movement is more random than targeted. Movement itself may be governed by control mechanisms which are known from other motile cells in the CNS (MERCER et al. 1994), and they probably follow tracks of "low tissue resistance" like perivascular spaces or white matter tracts when penetrating into densely packed neural tissue (EMMETT et al. 1991). Speed of movement, stop-and-go, and changes in direction are probably dictated by the extracellular matrix and the expression patterns of adhesion and cellular interaction molecules of both the migrating and the endogenous cells. If a browsing T cell arrives in close vicinity of a virus-infected site, movement becomes more specific and more directed, determined by a gradient of chemotactic factors. In the virus-infected brain, expression of many well known chemokines is up-regulated (ASENSIO and CAMPBELL 1997; MORIMOTO et al. 1996). It looks as if true homing to the virus-infected tissue is a rather late event in the course of T lymphocyte movement through the parenchyma.

How do T cells arrange in relation to virus-infected cells in the tissue? Very few studies on the dynamics and the spatial relationships between different lymphocyte subpopulations and virus-infected cells have been conducted so far. With the aid of computer-aided immunohistochemistry carried out at different times post-infection, we have analyzed these arrangements and their dynamic changes in the course of a viral CNS infection (DÖRRIES et al. 1991). Despite the fact that comparable amounts of $CD4^+$ and $CD8^+$ T lymphocytes can be recovered early after infection from the brain of coronavirus JHMV-infected rats, homing patterns of the two T cell subsets to virus infected areas differ. One week post-infection, with the onset of clinical symptoms, only a few $CD4^+$ T cells focus in these areas and they are outnumbered by far by accompanying $CD8^+$ T cells, which fill up the center of a virus-infected demyelinated plaque. A week later the picture changes completely. At the maximum of neurological disease expression, $CD8^+$ T cells are seen in intimate proximity to virus-infected cells, which form the border of the plaque. Numerous $CD4^+$ T lymphocytes have gathered in the area and the center of the plaque is filled with antibody-secreting plasma cells. Another week later, at the time of recovery, only few $CD8^+$ T lymphocytes are associated with the remainder of scattered virus-infected cells. The dominating population is clearly of the $CD4^+$ T cell subset. Over the entire observation period many macrophages/microglia concentrate in foci of T cell infiltration.

Comparable to the situation immediately after transmigration through the BBB, antigen presentation seems to play an important role in the spatial

arrangement of lymphocytes in virus-infected areas because strong up-regulation of class I and II major histocompatibility antigens is seen in JHMV-infected foci. Besides cells of hematopoietic origin, class I antigens are also expressed on the viral target cell, which in case of JHMV in rats is the oligodendrocyte. Class II antigens are up-regulated strongly on the brain-resident microglia cell. This was shown unequivocally by flow cytometry, which allows differentiation of brain-resident microglia from infiltrating macrophages/monocytes (Sedgwick et al. 1991b). This highlights the central role of MHC up-regulation in both the perivascular space and in the brain parenchyma for coordinated action and movement of T cells in the virus-infected brain. In this context the crucial question is what event causes up-regulation of MHC antigens in the virus-infected brain tissue? As pointed out above, IFN-γ is a strong up-regulating agent for MHC class I and class II antigens, and activated T lymphocytes, $CD8^+$ as well as $CD4^+$, are able to synthesize this cytokine abundantly. It is further known that expression of IFN-γ in the virus-infected brain is strongly dependent on a functional T cell compartment (Pearce et al. 1994). However, the tentative conclusion that MHC expression in the brain and homing of T lymphocytes to virus-infected areas are entirely dependent on IFN-γ secretion would pose the serious problem of the chicken and the egg: If homing of T lymphocytes is dependent on appropriate MHC expression, and up-regulation of these molecules is T cell dependent, it is difficult to understand how MHC molecules are up-regulated very early after CNS infection to guide infiltrating T cells to their targets. There are multiple observations which suggest that initial up-regulation of MHC antigens in the virus-infected brain is mediated by IFN-α/β and is independent from T lymphocytes. Pearce et al. (1994) noted that in nude mice which are intracerebrally infected by JHMV, MHC expression is as strong as in normal mice, even in the absence of detectable IFN-γ mRNA. Njenga et al. (1997) reported that IFN-α/β-receptor knockout mice have deficiencies in up-regulate of MHC class I antigens in the CNS soon after infection with Theiler's virus. Sandberg et al. (1994) observed that treatment of mice with anti-IFN-α/β antibodies reduced the inflammatory T cell reaction in the LCMV-infected brain. Finally, LCMV infection in IFN-γ knockout mice did not alter recruitment of T cells to the brain but had some consequences on the activation of macrophages (Nansen et al. 1998), and up-regulation of class I molecules on measles virus-infected glial cells is strongly associated with IFN-β (Dhib Jalbut et al. 1995). However, in this context it is very important to note that up-regulation of MHC class I antigens by IFN-β does not operate in neurons (Dhib Jalbut et al. 1995), although an antiviral state can be induced in these cells by IFN-β treatment (Ward and Massa 1995). Obviously, in neurons, strong IFN-γ signaling by inflammatory T cells and serious defects in the electrical properties of neurons are needed to allow up-regulation of class I molecules (see Sect. 2.1.1).

From these data it can be assumed that soon after infection, in the absence of infiltrating T cells, up-regulation of MHC antigens in the brain is strongly influenced by local production of IFN-α/β. Moreover, some viruses have been shown to induce MHC antigens (class I and class II) directly on brain-derived cells in the absence of IFN-γ (Gombold and Weiss 1992; Massa et al. 1986), or on brain

endothelial cells in a cytokine-independent manner (GAIRIN et al. 1991). Once T cells have settled in these areas they strongly enhance MHC expression on brain-resident cells (including defect neurons) by secretion of IFN-γ and thereby potentiate the inflammatory reaction. The necessity of appropriate MHC/viral antigen presentation to induce an inflammatory response by T cells in the virus-infected brain was very elegantly shown by DOHERTY and ALLAN (1986). Using bone marrow chimeras, it was demonstrated that, for a maximal T lymphocyte-mediated inflammatory response in the brain, it is necessary that MHC-restricted virus-specific T cells expand in peripheral lymphoid tissue must find virus infected cells in the CNS expressing the same MHC as seen in the periphery.

4.3 Profiles of Infiltration and Phenotypic Properties of T Lymphocytes

In the course of coronavirus JHMV-induced encephalomyelitis, a first peak of T lymphocytes can be recovered from the brain parenchyma a few days after intracerebral infection. This is followed by a second peak approximately 3 days later (DÖRRIES et al. 1991; HEIN et al. 1995; IMRICH et al. 1994). Infiltrating T cells can phenotypically be characterized by expression of the CD45RC molecule. CD45RC is an isoform of the common leukocyte antigen, which is expressed in the rat on unprimed T lymphocytes. Freshly activated T cells lose expression of this molecule but can re-express CD45RC when entering the state of a memory cell (BELL et al. 1998; SARAWAR et al. 1993). Using flow cytometry and immunostaining for CD45RC and the TCR, infiltrating T cells can be subdivided into two categories. Whereas in the first peak a significant amount of $CD45RC^+$, TCR^+ cells accompanies the majority of TCR^+, $CD45RC^-$ cells, the second peak of infiltration contains more than 98% TCR^+, $CD45RC^-$ cells. Thus, an initial influx of activated T lymphocytes ($CD45RC^-$) accompanied by a small amount of naive or memory T cells ($CD45RC^+$) is followed by a second wave of infiltrating T lymphocytes, which is composed almost entirely of T cells with an activated phenotype ($CD45RC^-$).

In addition to a state of fresh activation, infiltrating T cells show another remarkable feature in JHMV-induced encephalomyelitis. Virtually none of them express a receptor for interleukin 2 (IL-2r), which implies that they are in a state of terminal differentiation, no longer able to respond to proliferation-stimulating signals. Any attempt to induce proliferation in these cells either with antigen or a mitogen fails over a period of 3 weeks post-infection (IMRICH et al. 1994). Comparable data were reported from the EAE model, in which BMP-specific effector T cells stop proliferation when entering the brain parenchyma (OHMORI et al. 1992). Moreover, results from the Sindbis virus model of encephalitis in mice also suggest that T cells in the brain tissue have lost expression of the IL-2r and thereby their capability to respond to proliferation signals (IRANI et al. 1997).

What is the origin of the proliferation switch-off signal? Amongst several possibilities an intriguing hypothesis to explain T lymphocyte switch-off in the CNS

shall be discussed here: a strong and efficient antigen presentation on brain-resident professional APC-like microglia cells can send negative signals to tissue-infiltrating T cells which suppress the response to proliferative stimulation but which maintain effector functions of the T cell, as there is secretion of cytokines. There are multiple observations which lend support to this idea: (1) Application of BMP to the CNS without disturbing the BBB causes suppression of clinical EAE after transfer of BMP-specific T cell lines (HARLING BERG et al. 1991); (2) in contrast to peripheral macrophages, microglial cells are not able to induce proliferation and IL-2 secretion in $CD4^+$ T lymphocytes (FORD et al. 1995); and (3) although stimulating secretion of IFN-γ and TNF-α in $CD4^+$ T lymphocytes, MHC class II-positive microglial cells subsequently cause death in the contacting T cell population (FORD et al. 1996).

The nature of the negative signaling to T lymphocytes is not exactly defined. However, it is known that activation of tissue macrophages by TH1-cell-derived cytokines causes induction of the inducible nitric oxide synthetase (iNOS) which produces nitric oxide (NO) from L-arginine (for review see (KOLB and KOLB-BACHOFEN 1998)). Besides its static effects on infectious agents including viruses (KULKARNI et al. 1997; PERTILE et al. 1996), NO strongly suppresses the induction of proliferation in T lymphocytes (MILLS 1991) and may thereby be involved in the immunosuppressive effects of virus infections (BUTZ et al. 1994). In the brain, iNOS and NO are up-regulated after virus infection (AKAIKE et al. 1995; BI et al. 1995; HOOPER et al. 1995), and microglial cells have been shown to express iNOS in the presence of pro-inflammatory cytokines in HIV-infected patients (LANE et al. 1996) or during vesicular stomatitis virus (VSV) infection of the murine CNS (CHRISTIAN et al. 1996). Since up-regulation of iNOS occurs during acute viral CNS infection (OLESZAK et al. 1997), it seems possible that activated microglial cells contribute via NO to the inhibition of T cell proliferation in the CNS. If this assumption is correct, than microglial cells would fulfill a delicate regulatory role. On one hand, they act as stimulators of cytokine secretion in contacting T lymphocytes, which in turn enables them to assist in the elimination of infectious agents by enhanced phagocytosis and endosomal digestion. On the other hand, in a kind of feedback loop, they must prevent uncontrolled expansion of inflammatory T cells in order to avoid damage in the CNS.

4.4 Effector Mechanisms

4.4.1 $CD4^+$ T Lymphocytes

By virtue of the broad pattern of cytokines that can be secreted by $CD4^+$ T lymphocytes, these cells are central regulators of local immune effector functions. Dependent on the predominant cytokines secreted, $CD4^+$ regulator T cells are classified at least into four subpopulations, namely TH0–TH3. TH0 is an activated $CD4^+$ T cell which so far is not committed to differentiation along a certain pathway – a process that depends on external stimuli by cytokines. Under the

influence of IL-12, which is secreted predominantly by APC, a TH0 cell may develop into a pro-inflammatory TH1 cell, characterized by strong IFN-γ and TNF-α secretion. In the absence of IL-12 and in the presence of Il-4, TH0 cells can differentiate into anti-inflammatory TH2 cells, which predominantly act via secretion of IL-10 and help B lymphocytes to differentiate into antibody-secreting plasma cells. And finally, a less well understood differentiation pathway is that of TH3 cells, which, due to their secretion of TGF-β, are characterized as suppressor cells (HAFLER et al. 1997).

Analysis of the cytokine milieu in the inflamed brain of virus-infected animals has revealed expression of IFN-γ and/or TNF-α (IRANI et al. 1997; LANE et al. 1996; MOKHTARIAN et al. 1996; MORRIS et al. 1997; PEARCE et al. 1994; WESSELINGH et al. 1994), a finding which implies a TH1-determined cytokine milieu. Antigen-presenting microglial cells can stimulate secretion of IFN-γ in $CD4^+$ T lymphocytes (FORD et al. 1996), and respond to IFN-γ by up-regulation of MHC I antigens (GRAU et al. 1997) and the CD40 molecule (NGUYEN et al. 1998), as well as by proliferation (SEDGWICK et al. 1998). Up-regulation of the CD40 molecule could allow interaction with activated T cells via the CD40 ligand, which on tissue resident macrophages usually results in the secretion of multiple pro-inflammatory substances like TNF-α, IL-12, macrophage inflammatory protein (MIP)-1α and NO (STOUT and SUTTLES 1996). Indeed, it has been shown that activated microglia can secrete these substances (ALOISI et al. 1997; FREI et al. 1987; HAYASHI et al. 1995) and, since they are better stimulators of TH1 cells than of TH2 cells (ALOISI et al. 1998), an increasing pro-inflammatory micro-environment will arise in areas where $CD4^+$ T cell/microglia interactions take place. All of these pro-inflammatory substances act as attractants for peripheral blood leukocytes, either directly as chemoattractants or indirectly by up-regulation of adhesion molecules such as ICAM-1 on adjacent endothelial cells. Additionally, an enhanced capability of antigen processing and presentation is achieved by the responding tissue macrophages, as shown by a strong up-regulation of MHC class II and class I molecules as well as of B7 molecules (MENENDEZ IGLESIAS et al. 1997).

All of these observations define a role for $CD4^+$ TH1 cells as potent activators that switch the immunologically calm CNS to a state of competent immunological reactivity. As a consequence of this regulatory circuit, more and more inflammatory cells including peripheral macrophages, mobilized microglial cells, $CD8^+$ T cells, and antibody-secreting plasma cells are trapped in virus-infected sites. With increasing activation of the microglia, however, negative regulatory effects on T lymphocytes may overcome positive signals of attraction and stimulation of effector functions. In particular, the release of NO and the overexpression of B7 molecules can result in T cell arrest or death (see Sect. 4.3), and chronic and strong stimulation of T lymphocytes by antigenic recognition up-regulates the CD95/FasL receptor system on these cells (LYNCH et al. 1995).

Very recently, published investigations support the concept that, besides regulating effects on the influx and homing of peripheral immune system cells, the presence of $CD4^+$ T effector lymphocytes is indispensable for $CD8^+$ T lymphocytes to exert their effector functions, namely, reducing the viral load of the

brain by destruction of virus-infected cells and/or by inhibition of intracellular viral growth. As shown in coronavirus JHMV infected mice, clearance of virus from the CNS by $CD8^+$ T lymphocytes, but not their recruitment to the tissue, is dependent on the presence of a functional $CD4^+$ T lymphocyte compartment in these animals (STOHLMAN et al. 1998). Comparable data were described in BDV-infected rats. Here, it was demonstrated that transfer of primed $CD4^+$ T cells, which were not cytotoxic themselves, resulted in many more $CD8^+$ cytotoxic T cells in the brain of BDV-infected animals than in non-transferred animals (PLANZ et al. 1995).

Although the molecular basis of $CD4^+$ and $CD8^+$ T cell interactions is still unknown, studies on the role of CD95/FasL in $CD4^+$ and $CD8^+$ T lymphocyte cross-talk offers some interesting perspectives (LYNCH et al. 1995). It is known that CD95/FasL interaction can exert both stimulating and death-inducing effects on lymphocytes, dependent on the stage of differentiation of interacting cells. Freshly activated $CD4^+$ T cells rapidly up-regulate both CD95 and FasL. However, whereas CD95 expression is induced on both T cell subsets, TH1 and TH2, FasL is seen only on the TH1 T cell subset. Engagement of these receptors on freshly activated cells does not induce apoptosis but enhances proliferation and cytokine secretion (IFN-γ, IL-2 and TNF-α). TH1 cells expressing FasL can also interact with the CD95 molecule expressed on precytotoxic $CD8^+$ T lymphocytes when both cell subsets make contact with an APC cell via their antigen receptor. This FasL/CD95-mediated $CD4^+/CD8^+$ T lymphocyte cross-talk drives differentiation of the $CD8^+$ T lymphocyte into an activated, MHC class I-restricted killer cell in an IL-2 independent way. Although it is completely unknown if these mechanisms also operate in non-lymphoid tissue like the CNS, observations of STOHLMAN et al. (1998) support this idea. They reported that apoptosis of virus-specific $CD8^+$T lymphocytes in the CNS of JHMV-infected mice is considerably higher in animals that were depleted of $CD4^+$ T lymphocytes. It seems that $CD4^+$ T lymphocytes help $CD8^+$ T effector lymphocytes to survive and that they are important regulators of $CD8^+$ T-lymphocyte-mediated killing. This might explain why clearance of the virus from the brain by $CD8^+$ T lymphocytes is $CD4^+$ T-lymphocyte-dependent, or at least substantially enhanced by TH1 $CD4^+$ T lymphocytes.

4.4.2 $CD8^+$ T Lymphocytes

$CD8^+$ T lymphocytes can kill virus-infected cells by at least two mechanisms which, although acting on different signal pathways, both will end in the induction of the apoptotic killing machinery of the affected target cell. The perforin pathway acts via pore formation in the membrane of the target cell by perforins and transfer of granzyme B (GrB) through this pore into the cytoplasm of the target cell. Here, GrB activates and converts pro-caspases to active caspases, which are mediators of apoptosis. The FasL/CD95 pathway acts via engagement of the CD95 molecule on the target cell with the FasL expressed on the killing $CD8^+$ T cell. Upon engagement, a stimulating signal is transduced by the CD95 molecule via intracellular death domain proteins, which convert pro-caspases into death-inducing caspases (FROELICH et al. 1998) in the virus-infected cell.

There is no doubt that $CD8^+$ T cells extracted from the virus-infected CNS can kill virus-infected cells in vitro in a MHC class I-restricted manner (HEIN et al. 1995; PLANZ et al. 1993). This idea is also supported by in vivo data showing a disastrous clinical course of viral CNS infection in animals supplemented with virus-specific $CD8^+$ T cells at the time of virus infection. From this experience and the results from several in vivo manipulations of $CD8^+$ T lymphocytes, e.g. depletion of $CD8^+$ T cells (BILZER and STITZ 1994; SUBAK SHARPE et al. 1993), CNS infection in β2-microglobulin- or perforin-deficient mice (ROSSI et al. 1998), and constitutive transgenic expression of MHC class I molecules in neurons (RALL et al. 1995), it was concluded that $CD8^+$ T cells indeed can kill virus-infected brain cells in vivo. In the case of neurons, however, the cell must be seriously damaged by the virus infection before MHC class I expression is up-regulated and T cell-mediated killing can take place (see Section 2.1.1). If this happens, in vitro experiments from Rensing Ehl's group suggest that the perforin-dependent pathway is preferred by $CD8^+$ T effector cells (RENSING EHL et al. 1996).

Despite this strong but indirect experimental support of the idea that $CD8^+$ T lymphocytes kill cells in the brain parenchyma, there are a substantial data which suggest that this is the exception rather than the rule. In various animal models $CD8^+$ T lymphocytes extracted from the virus-infected brain cannot kill ex vivo; rather, they have to be restimulated with strong signals, sometimes including allo- or even xenogenic TCR engagement, before substantial killing activity can be detected on virus-infected syngeneic target cells (HEIN et al. 1995). Work from KÜNDIG et al. (1993) helps to explain the virus-cleansing property of T lymphocytes in the CNS by soluble factors rather than by killing. After immunization of mice with VSV, the animals were challenged with two different recombinant vaccinia viruses (vacc) one expressing the nucleoprotein of VSV (vacc-VSV-NP) and the other expressing the glycoprotein of LCMV (vacc-LCMV-GP). Surprisingly, animals challenged with the VSV-unrelated vacc-LCMV-GP could restrict growth of a vacc-LCMV-GP in the brain, but not in the testis or ovaries. This bystander effect in the CNS was partly inhibited by treatment with anti-IFN-γ antibodies. This effect was seen in mice with the haplotype $H\text{-}2^b$, which usually clear LCMV by $CD8^+$ T lymphocytes, as well as in animals with the haplotype $H\text{-}2^k$, which use $CD4^+$ T cells for LCMV clearance.

Non-cytolytic clearance of virus by MHC class I-restricted T cells seems to be the regular effector mechanism if viruses persist in neurons without causing lethal damage to their host cells. Work from Oldstone's laboratory described removal of virus from persistently infected neurons after adoptive transfer of virus-specific cytotoxic T cells (OLDSTONE et al. 1986; TISHON et al. 1993). Although lymphoid infiltration was noted in the brain parenchyma and viral load was reduced considerably, no neuronal loss occurred. Most remarkably, clearance of virus by non-lytic mechanisms is limited to the CNS and takes much longer than in peripheral organs, where tissue destruction and inflammation can be seen. The mechanism of this elimination process remains to be unraveled.

Cumulative data suggest that $CD8^+$ T lymphocytes can successfully combat virus-infections in the brain. The strategy, however, seems to differ from that

followed in peripheral infections. Due to the lethal hazard that comes from irreversible destruction of neurons, $CD8^+$ T lymphocytes try to cure the cell instead of killing it, at least in the case of neurons which are functionally not impaired by the virus (concerning Ig-induced clearance of viruses from neurons, see chapter by Dietzschold et al.).

4.5 Downsizing of T Effector Lymphocytes

To date, there are no convincing data which would suggest that T cells entering the CNS ever leave this organ. It seems very likely that all undergo apoptosis and that apoptotic cells are phagocytozed by brain-resident microglia cells. So far, the death-triggering signal(s) are poorly defined in the virus-infected CNS.

CD95/FasL-mediated apoptosis might work in a way similar to that reported for the inflamed EAE brain (Sun et al. 1998). In EAE, glial as well as inflammatory $CD4^+$ T lymphocytes express both CD95 and FasL. This situation allows a two-way induction of apoptosis. Not only do T lymphocytes destroy glia cells, but glial cells can kill effector T lymphocytes. The regulatory events that influence the direction of killing is so far not entirely clear, but differential regulation of the density of death receptors on both cell populations could play a decisive role.

Theoretically, CD95/FasL-mediated killing might happen amongst T cells themselves. As discussed above, all activated T lymphocytes co-express CD95 and FasL. As long as the density of virus-infected target cells is high, it is unlikely that T cells meet and kill each other by CD95/FasL interaction. However, with increasing clearance of virus-infected cells from the tissue, the risk of contacting another CD95/FasL-expressing T lymphocyte increases, which could finally result in mutual killing of effector T lymphocytes. However, since expression of the CD95/FasL molecules is strictly dependent on continuous antigenic stimulation, these dangerous molecules will be down-regulated along with the decreasing number of virus-infected target cells, thereby probably allowing some T cells to survive.

Even more speculative than the CD95/FasL hypothesis is the idea that differential expression of T cell costimulatory molecules like B7 could contribute to T cell death in the brain. Engagement of the B7/CD 152 receptor/ligand pair principally causes abrogation of IL-2 synthesis in T cells. The consequences of this switch-off signal depend on the state of T cell differentiation. In resting non-activated T cells, B7/CD 152 interaction causes cell cycle arrest; in activated proliferating T lymphocytes, which depend on IL-2 for growth, apoptosis occurs after B7/CD 152 interaction (Scheipers and Reiser 1998). The death signal in the proliferating T cell is independent of CD95/FasL engagement. In Theiler's virus infection of the CNS, B7 is expressed in the virus-infected tissue on all MHC class II-positive cells including brain-infiltrating macrophages and T cells as well as brain-resident microglia (Pope et al. 1998).

In the model of EAE, Bauer et al. (1998) reported consequent elimination of inflammatory T lymphocytes in the parenchyma but not in connective tissue compartments of the brain. This process is totally independent from any antigen

specificity. Since apoptosis of T cells in the brain occurs also in mice deficient in the CD95 gene (MALIPIERO et al. 1997), and apoptosis of T cells can be induced by microglial cells also in the absence of B7 expression (FORD et al. 1996), it is very likely that, besides CD95/FasL or B7/CD 152, other mechanisms of T cell elimination operate in the brain parenchyma with so far undefined signal pathways.

Whatever causes death of T lymphocytes in the brain, the CNS plays an active part in this process of elimination. With the dying T cells, the CNS-localized immune response terminates and the tissue returns to its relative immunological silence.

5 Consequences of T Cell Action

After having discussed how the T cell compartment is sensitized to viral antigens from brain, how these cells enter the CNS and home to virus-infected areas, and how they are eliminated, the consequences of T lymphocyte-mediated inflammation need to be examined. Many contradictory data have been published on this topic in the past, which roughly can be grouped into two opposite positions: (1) T cell action in the brain is protective and (2) T cell action in the brain is destructive and disease-inducing. Of course, careful evaluation of all published data reveal a much more complex and differentiated picture, which will be discussed in this chapter. Many routes were followed to make the important distinction between virus-induced and T lymphocyte-mediated destruction of CNS cells. The basis of most experimental approaches to this question was infection of animals that were genetically immunodeficient or that were immunocompromised by treatment with drugs or antibodies that interfere with immune system function.

The clinical consequences of T cell action in the virus-infected brain depend largely on: (a) What is the state of T cell differentiation in relation to the kinetics of viral spread in the tissue? (b) What type of non-T cell immune effector cell acts shortly before or concomitantly with T cells in tissue?

Both points are strongly influenced by the genetic background of the infected host. In general, it can be concluded that a rapid recruitment of a virus-specific T lymphocyte response to the CNS tissue, which is accompanied by virus-neutralizing antibody-secreting plasma cells, will cause much fewer clinical complications than a delayed and rather unspecific response. In the latter case it might even be less harmful to the host if no T cell response at all is recruited to the brain. A typical example demonstrating these rather complicated interrelationships is the experimental infection of the rat inbred strains Lewis and Brown Norway with coronavirus JHM (IMRICH et al. 1994; SCHWENDER et al. 1991). Brown Norway rats never showed any clinical signs of the infection, despite the fact that virus replicates in the brain. Infected rats showed a very early and specific T lymphocyte response in the CLNs and very rapidly recruited a strong virus-neutralizing antibody response to the brain. Consequently, extracellular viral spread was restricted

efficiently, allowing a limited number of T lymphocytes to clear the virus from a few, small virus-infected foci in a subclinical manner. In contrast, Lewis rats responded in the early phase of the infection with a broad expansion of T cell clones in CLNs but a delayed and weak virus-neutralizing antibody response. This delay allowed the virus to spread throughout the CNS, including the spinal cord. The immune system responded with a strong infiltration of T lymphocytes ($CD4^+$ and $CD8^+$). $CD8^+$ T cells are MHC class I-restricted killers and, due to the widespread infection of the brain, $CD8^+$-mediated clearance of virus-infected cells contributes to neurological disease. These effects are enhanced by $CD4^+$ T lymphocytes, because they are of the TH1 type and thus attract inflammatory accessory cells like monocytes from the periphery. Many animals die within 7–10 days post-infection, but roughly 60% start to recover at about 12 days post-infection. In theses animals a weak but obviously sufficient virus-neutralizing antibody response can be detected in the CNS. From these data one would assume that the action of T lymphocytes in CNS tissue generally is dangerous for the host. Whether T cell action causes neurological disease depends largely on the quantity of recruited cells, which in turn is related to the extent of viral spread in the tissue. In other words, any mechanism which keeps the virus from spreading warrants a subclinical effector phase of T lymphocytes.

Recently, we have added further support to this idea by irradiation of Lewis rats followed by reconstitution of the immune system with naive spleen and lymph node cells, which were depleted from individual lymphocyte subsets (Schwender et al. 1999). These partially deficient and immunologically naive animals were infected by JHMV and the development of neurological disease was monitored for the following 9 days. It turned out that, whenever B lymphocytes were absent from the transferred lymphocytes, the animals very rapidly became moribund within a few days. Infection of incompetent and not-reconstituted animals, however, resulted in a mild onset and progression of disease that in its severity was well below the level of T cell-reconstituted animals.

At first glance, these data contradict many reports which unequivocally showed that transfer of T lymphocytes protects from virus-induced neurological disease (Erlich et al. 1989; Körner et al. 1991; Stohlman et al. 1995; Sussman et al. 1989; Yamaguchi et al. 1991). A more detailed look discloses that in all these cases primed T lymphocytes, T cell lines or T cell clones with specificity for the challenging virus were transferred before infection. As a result, the virus was faced with an overwhelming amount of highly differentiated T effector cells, which immediately interfere with viral spread. If, as in the case of a naive T cell compartment, the race between the virus and the defending T cell system starts with entry of the virus, the amount of time needed by the host to differentiate effector T cells determines the clinical outcome. This is also supported by experiments in which virus-specific T cell lines were transferred at different time points before or after viral infection. Virus-specific T lymphocytes are protective when given shortly before or concomitantly with the virus. Adoptive transfer of such cells 2 days after infection causes severe enhancement of neurological disease (Yamaguchi et al. 1991).

Taken together, T effector lymphocytes are a two-edged sword for the host. If they act soon after infection, they will most likely help to prevent disease. If their recruitment is delayed and a virus-neutralizing antibody response is absent, they can contribute significantly to disease.

6 Summary

T lymphocytes play a decisive role in the course and clinical outcome of viral CNS infection. Summarizing the information presented in this review, the following sequence of events might occur during acute virus infection: After invasion of the host and a few initial rounds of replication, the virus reaches the CNS in most cases by hematogeneous spread. After passage through the BBB, CNS cells are infected and replication of virus in brain cells causes activation of the surrounding microglia population. Moreover, local production of IFN-α/β induces expression of MHC antigens on CNS cells, and microglial cells start to phagocytose cellular debris, which accumulates as a result of virus-induced cytopathogenic effects. Upon phagocytosis, microglia becomes more activated; they up-regulate MHC molecules, acquire antigen presentation capabilities and secrete chemokines. This will initiate up-regulation of adhesion molecules on adjacent endothelial cells of the BBB. Transmigration of activated T lymphocytes through the BBB is followed by interaction with APC, presenting the appropriate peptides in the context of MHC antigens. It appears that $CD8^+$ T lymphocytes are amongst the first mononuclear cells to arrive at the infected tissue. Without a doubt, their induction and attraction is deeply influenced by natural killer cells, which, after virus infection, secrete IFN-γ, a cytokine that stimulates $CD8^+$ T cells and diverts the immune response to a TH1-type $CD4^+$ T cell-dominated response. Following the $CD8^+$ T lymphocytes, tissue-penetrating, TH1 $CD4^+$ T cells contact local APC. This results in a tremendous up-regulation of MHC molecules and secretion of more chemotactic and toxic substances. Consequently an increasing number of inflammatory cells, including macrophages/microglia and finally antibody-secreting plasma cells, are attracted to the site of virus infection.

All trapped cells are mainly terminally differentiated cells that are going to enter apoptosis during or shortly after exerting their effector functions. The clinical consequences and the influence of the effector phase on the further course of the infection depends on the balance and fine-tuning of the contributing lymphoid cell populations. Generally, any delay in the recruitment of effector lymphocytes to the tissue or an unbalanced combination of lymphocyte subsets allows the virus to spread in the CNS, which in turn will cause severe immune-mediated tissue effects as well as disease. If either too late or partially deficient, the immune system response may contribute to a lethal outcome or cause autosensitization to brain-specific antigens by epitope spreading to the antigen-presenting system in peripheral lymphoid tissue. This could form the basis for subsequent booster reactions of

autosensitized $CD4^+$ T cells – a process that finally will end in an inflammatory autoimmune reaction, which in humans we call multiple sclerosis. In contrast, a rapid and specific local response in the brain tissue will result in efficient limitation of viral spread and thereby a subclinical immune system-mediated termination of the infection.

After clearance of virus-infected cells, downsizing of the local response probably occurs via self-elimination of the contributing T cell populations and/or by so far unidentified signal pathways. However, much of this is highly speculative, and more data have to be collected to make decisive conclusions regarding this matter.

Several strategies have been developed by viruses to escape T cell-mediated eradication, including interference with the MHC class I presentation pathway of the host cell or "hiding" in cells which lack MHC class I expression. This may result in life-long persistence of the virus in the brain, a state which probably is actively controlled by T lymphocytes. Under severe immunosuppression, however, reactivation of viral replication can occur, which is a lethal threat to the host.

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