Immune response to retroviral infections of the brain

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TABLE OF CONTENTS

# 1. ABSTRACT

Various neurological manifestations of retroviral infections have been reported, including peripheral neuropathy, encephalopathy and neuronal degeneration. After penetration into the central nervous system (CNS) the invading retroviruses meet a unique immunological situation that differs significantly from that in the periphery. Due to the blood-brain barrier with its general access restrictions peripheral T-cells, monocytes and B-cells are only "guests" in the brain; instead the immune balance is shifted in favour of the local innate immunity with microglia, astrocytes, cytokines/chemokines and complement forming the dominating defence network. The present article focuses on the most important retroviral infections and highlights the immunological aspects of the neuropathogenesis induced by selected retroviruses. These aspects include: (i) local and infiltrated immune cells as targets of retroviral infection; (ii) stimulation of the cerebral immunity network by retroviruses and subsequent steps of antiviral defence; and (iii) immune activation products as potential contributors to neural damage in the sensitive brain tissue.

# 2. INTRODUCTION

## 2.1. Cerebral infection by members of the Retroviridae family

Retroviral infection can cause several types of neuropathological processes, such as intracerebral hemorrhage, spongiform encephalopathy, demyelination, neuronal degeneration and inflammation. Representatives from all three retrovirus subfamilies are known to penetrate into the CNS with varying consequences. Spumaviruses have been detected in the brain but not been associated with any neurological dysfunction, although they induce foamy changes in cells *in vitro*. Therefore they will not be discussed here further. Within the Oncornavirinae subfamily murine leukaemia viruses (MuLV) and human T-cell leukaemia virus 1 (HTLV-I) induce neuronal damage. The Lentivirinae subfamily harbors the greatest number of neurotropic viruses with Visna virus, human immunodeficiency virus (HIV) and its relatives simian immunodeficiency virus (SIV) and feline immunodeficiency virus (FIV). The precise pathogenic mechanism remains elusive for all retroviruses and is object of extensive studies. In most cases, infection of neurons is

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**Table 1**. Interaction of Oncornavirinae and Lentivirinae with cerebral immune response

| | Infected immune cells in CNS | Mechanisms for neural degeneration | Altered cytokine pattern associated with neurodegeneration |
|---|---|---|---|
| **Murine leukaemia virus** | | | |
| Moloney MuLV | Microglia | Microglial activation/dysfunction<br>Aberrant cytokine production | TNF-$\alpha$<br>IL-6 |
| Friend MuLV | Microglia | Microglial activation/dysfunction<br>Aberrant cytokine production | |
| **Human T-cell leukaemia virus** | | | |
| HTLV-I | Infiltrating CD4$^+$ and CD8$^+$ cells<br>Astrocytes<br>Microglia | Direct attack of hyperactive CTL<br>Tax-induced perturbation of cellular protein synthesis pattern<br>Aberrant cytokine production | IFN-$\gamma$<br>TNF-$\alpha$<br>IL-6<br>IL-1$\beta$<br>IL-2 |
| **Immunodeficiency viruses** | | | |
| FIV | Infiltrating macrophages<br>Microglia<br>Astrocytes | Microglial activation/dysfunction<br>Aberrant cytokine production | TNF-$\alpha$<br>IL-1$\beta$ |
| HIV, SIV | Infiltrating macrophages and CD4$^+$ cells<br>Microglia<br>Astrocytes | Microglial activation/dysfunction<br>Aberrant cytokine production<br>Secretion of Nef, gp120 by infected astrocytes and microglia<br>Increased complement synthesis and chronic activation | IFN-$\gamma$<br>TNF-$\alpha$<br>IL-4<br>IL-10<br>TGF-$\beta$<br>IL-1$\beta$<br>IL-6 |

not or only marginally observed, suggesting an indirect mechanism of neuronal dysfunction.

## 2.2. The immune network within the CNS

Invading retroviruses are confronted with a unique immunological landscape in the brain to which they are not adapted by their former residence in the periphery of the host. However, the brain is an immunopriviledged site with an extremely low spontaneous immune activity and potent immunosuppressive effectors, thus facilitating microbial survival in this environment.

Key players that belong to the defence arsenal of the brain against invading microbes are resident immune cells (microglia, astrocytes) and soluble factors (complement, cytokines/chemokines). These components carry the main burden of antiviral defence, particularly in early stages of cerebral infection, since the blood-brain barrier restricts the access of the peripheral immunity. In the later course of infection, the local CNS immunity is supported by infiltrating immune cells from the periphery like CD4$^+$ and CD8$^+$ T-cells and monocytes/macrophages; in addition leakage of the blood-brain barrier might allow diffusion of peripheral cytokines/chemokines and complement into the brain.

Working together as a network, local and infiltrating immune components, either cellular or soluble, mean well to fight against microbial invaders, aiming to reconstitute the homeostasis in the infected brain. However, despite this good intention, the brain as an extremely sensitive organ may be damaged by the subsequent immune reaction and inflammation. This sensitivity creates the necessity to include both inflammatory and anti-inflammatory mediators in the antiviral defence. Our review aims to highlight the complex interactions between retroviral agents and cerebral immunity, including (i) immune cells as target for retroviral infection with subsequent loss of their functionality and capacity to maintain brain homeostasis and the establishment of a

persistent retroviral reservoir; this has been demonstrated both for infiltrating monocytes and T-lymphocytes as well as for resident microglia and astrocytes; (ii) stimulation of an antiviral immune concert, made up by the network of local and infiltrating participants; and (iii) the potentially detrimental effect of excessive immune activation, exerting a direct harmful or even toxic effect on neurons, or profoundly disturbing the brain homeostasis with an indirect effect on sensitive neurons. These aspects of retroviral infection of the CNS are also shortly summarized in Table 1.

## 2.2.1. Microglia

Parenchymal microglia represent the most important resident immune cell type within the CNS and fulfil a variety of immunological functions like phagocytosis, antigen presentation, cytokine production and complement synthesis. Furthermore they act as central regulators of inflammation, cellular activation and adaptive immune response (1,2). They represent the main local target cell for retroviral infection of the CNS.

Under physiological conditions, parenchymal microglia present themselves in a ramified quiescent state and lack significant immunological activity. However, upon infection or brain insult they change to a more rounded phenotype and rapidly undergo a complex activation cascade (2). This process follows a stereotypic pattern (3): (i) stage of alert with increased expression of immune molecules; (ii) homing stage with adhesion to damaged structures, intense cell proliferation, high expression of inflammatory mediators (cytokines, oxygen intermediates), and molecules of the antigen presentation; (iii) transformation into phagocytes with phagocytic activity towards invading pathogens and the ability of potent antigen presentation to activate infiltrating T-cells (4). Microglial activation contributes to limit microbial spreading or even clear the cerebral infection. Furthermore, the activation strengthens the neuroprotective capacity of

microglia by upregulation of scavenger molecules, glutamate transporters to remove excessive neurotransmitter, and neurotrophins for regeneration of damaged neurons. However, activated microglia secrete effector molecules (proteases, oxide radicals, cytokines), which are potentially harmful for the vulnerable neurons (2).

A variety of cell-cell interactions and soluble molecules regulate the activation status of microglia with stimulatory or inhibitory signals. Neurons as well as astrocytes are able to suppress the activation of microglia (5,6). In contrast interaction between microglia and activated T-cells triggers bidirectional stimulation as it promotes microglial inflammatory responses and provides a strong costimulatory signal to the lymphocytes (7). Similarly, the complement activation products C3a and C5a induce microglial activation via binding to their corresponding receptors (8). A network of pro- and anti-inflammatory cytokines and chemokines also modulates the microglial functionality, with regulation of antigen-presentation, migration, proliferation and synthesis of other cytokines/chemokines (9). Microglia are even able to sense peripheral inflammation and to adopt a preactivated state that enables rapid reaction after microbial penetration into the CNS (2,9).

### 2.2.2. Astrocytes
Astrocytes are the most numerous cell type in the brain and exert a broad functional spectrum. Being an integral part of the blood-brain barrier they inhibit the penetration of most pathogens into the brain. Their activities to maintain brain homeostasis are absolutely necessary for neuronal survival (10). Astrocytes are a general target for retroviral infection, although to a lesser extent than microglia.

Astrocytes also fulfil a number of immunological functions (2). They are able to produce most components of the complement system (11); expression of complement receptors renders astrocytes sensitive for the numerous biological effects of complement fragments. Astrocytes are also a main producer of both inflammatory and anti-inflammatory cytokines and chemokines in the brain (12). In addition, astrocytes keep the brain an immunoprivileged site by suppression of inflammatory activation of other immune cells. The contact with astrocytes maintains microglia in a dormant state, inhibits phagocytosis and downmodulates their expression of MHC molecules and proinflammatory cytokines (2). Similarly, astrocyte-derived soluble factors deactivate infiltrating monocytes and reduce the synthesis of adhesion and antigen presenting molecules (13). Astrocytes are considered to be semi-professional antigen-presenting cells; their activation upregulates the production of MHC and costimulatory molecules and thus allows antigen presentation to lymphocytes (14,15). Activated astrocytes can even achieve the capacity of phagocytosis, although they are less potent than microglia (16).

### 2.2.3. Complement
Complement is a universal defence system which fights against a large diversity of pathogens and guarantees an immediate and potent limitation of infection (17). Complement comprises a set of soluble proteins arranged as an activation cascade of interacting components which finally results in the generation of a lytic pore in the membrane of the pathogen or the infected cell. Most brain cells also express complement receptors thus being responsive towards the broad biological activity of complement products, including chemotaxis, cytokine induction and cell activation (11). However, chronic complement activation and enhanced synthesis is associated with neural damage, as shown for various neurodegenerative diseases like Alzheimer's disease and Multiple Sclerosis (11). This damage might be mediated by complement-dependent bystander lysis since neurons which express only limited amounts of the membrane-bound negative regulators of complement activation are extremely sensitive against complement attack (18). Furthermore, complement activation might result in oxidative stress through stimulation of respiratory burst activity by complement-derived fragments.

Although the cerebral expression of complement is low under physiological conditions the synthesis is upregulated either directly by pathogen contact or indirectly by inflammatory cytokines (11). IFN-$\gamma$ is the most effective cytokine to enhance complement synthesis by astrocytes, microglia and neurons (19), but also other cytokines like TNF-$\alpha$, IL-1$\beta$ and IL-8 are potent inducers (20).

### 2.2.4. Cytokines and chemokines
Cytokines and chemokines are small polypeptides with pleiotropic activity, partly overlapping, partly synergistic or antagonistic. They represent a central mean of crosstalk between resident but also infiltrated immune cells in the brain. Neurons, astrocytes, microglia and oligodendrocytes as well as infiltrated monocytes and lymphocytes are important producers of these molecules in the brain. Cytokines/chemokines mutually regulate their synthesis which is also modulated by additional factors like brain lesions or presence of invading pathogens (3). All types of brain cells possess receptors which provide responsiveness for a large variety of different cytokines/chemokines (21). The various functions of cytokines and chemokines include induction or suppression of inflammation, chemoattraction of peripheral cells or resident immune cells to the site of lesion or infection, activation or deactivation of cells, induction of apoptosis and modulation of the cellular expression pattern. Some of the proinflammatory cytokines are also discussed to be neurotoxic like tumor necrosis factor-$\alpha$ (TNF-$\alpha$) and interferon-gamma (IFN-$\gamma$).

## 3. IMMUNOLOGICAL ASPECTS OF RETROVIRAL INFECTION OF THE CNS

### 3.1. Oncornavirinae
### 3.1.1. Murine leukaemia viruses (MuLV)
Infections with murine leukaemia viruses result in peripheral immunological abnormalities such as T-cell and B-cell dysfunctions, hypergammaglobulinemia and splenomegaly. When the CNS is affected ecotropic

oncornavirinae induce a spongiform encephalomyelopathy primarily affecting motor nuclei and motor cortex of the brain and the spinal cord; corresponding neurological symptoms are tremor, ataxia, hind limb paralysis, impaired spatial learning and hyperexcitability (22). Members of this neurotropic MuLV group are wild-type CasBrE, several variants of Friend MuLV, and mutants of Moloney MuLV (MoMuLV) (23,24).

Microglial infection and activation as well as alteration of cytokine levels imply a fundamental role of immune reactions in the neuropathogenesis of murine leukaemia viruses. Cerebral infection of mice with ts1, a mutant of MoMuLV which has acquired the ability to cause neuronal cell death through mutations in its env gene, results in a neuroimmunodegenerative syndrome (25) that resembles AIDS in some aspects (26,27). Microglia are the primary cellular target for ts1; the neuronal degeneration caused by ts1 is likely linked to microglial activation and subsequent release of cytokines and cytotoxins which affect neighbouring motor neurons (27). This fits together with former results showing that the neurovirulence of ts1-infected mice correlates with the ability of the virus to infect, replicate in, and activate resident microglia (28). Putative microglia-derived factors are the cytokines TNF-$\alpha$ and interleukin-6 (IL-6) which were shown to be present in higher tissue concentrations in the CNS of ts-1 infected than in noninfected mice (29). TNF-$\alpha$ is a pro-inflammatory cytokine, the receptors for which are expressed on neurons and glial cells throughout the CNS (30). The immunomodulatory as well as the neurotoxic and neuroprotective effects of TNF-$\alpha$ depend on the pathophysiological context, the presence of receptors on target cells, co-expressed factors and the compartment of its release. Cerebral TNF-$\alpha$ expression is associated with the severity of the ts1-induced neuropathology, and the levels of TNF-$\alpha$ are elevated within the spongiform lesions of the ts1-infected CNS (25). A possible mechanism of TNF-$\alpha$-induced neurotoxicity and degeneration may be direct killing of neurons by binding to the receptors and subsequent activation of apoptotic caspases. Indirect mechanisms may be the up-regulation of neurotoxic proteins (other cytokines or chemokines) or the induction of excitotoxins (glutamate, L-cysteine) in glial cells, thus offering a lethal excitotoxic environment to neurons (31).

Microglia were also identified by double immunostaining to be the predominant viral target for Friend MuLV (32,33), thus implying that microglia dysregulation and subsequent aberrant cytokine production after infection might be a general mechanism for retroviral neuropathogenesis.

### 3.1.2. Human T-cell leukaemia virus

HTLV-I is endemic in southern Japan, the Caribbean, Africa and some parts of the United States. It is estimated that 10-20 million people worldwide are infected with HTLV-I. Whereas the majority of infected individuals are asymptomatic carriers, 5-10% develop a disease, either adult T-cell leukaemia or chronic HTLV-associated myelopathy (HAM), also known as tropical spastic paraparesis (TSP) (34). The close relative HTLV-2 has not

been definitely linked to any neurological disease and will not be dealt here further.

Clinically, HAM/TSP is a slowly progressive neurological condition, characterized by chronic spastic paraparesis with weakness and spasticity of the extremities, mild peripheral sensory impairment, hyperreflexia and sphincter symptoms. Neuropathological findings are demyelinated lesions in brain and spinal cord as well as chronic inflammation with infiltration of lymphocytes and macrophages into the CNS. Detailed electron microscopic analysis of the lesions revealed disintegration of myelin sheaths up to completely demyelinated axons, axonal degeneration and glial scarring (35).

Convincing evidence suggests that immune activation and immunologic dysregulation/abnormalities contributes to the histopathological changes in TSP/HAM. The immune response in HAM/TSP patients may be a typical example for the transition from a beneficial response aimed at controlling viral replication, to a detrimental reaction that contributes to the neuropathology of this viral infection. Potentially useful and at the same time harmful immune elements and parameters include CD4$^+$ cells, CD8$^+$ cells, astrocytes, high antibody titers and a variety of inflammatory cytokines/chemokines, which together form an interactive network.

During the peripheral infection CD4$^+$ T-lymphocytes, the conductors of the peripheral immune network, are the primary cellular target for HTLV-I in the blood (36). After infection these cells spontaneously proliferate and express pro-inflammatory cytokines and adhesion molecules that allow the CD4$^+$ lymphocytes to infiltrate the CNS (37). CD8$^+$ lymphocytes, the functionally cytotoxic T-lymphocytes (CTL) which recognize viral antigens in the context of HLA class I, represent a further viral reservoir. Thus both cell types might transport the virus into the brain and trigger neurological dysfunctions.

Furthermore, both infiltrated CD4$^+$ and CD8$^+$ T-cells are hypothesized to contribute directly to neuroinflammation and neuronal damage. The inflammatory lesions in the infected CNS of HAM/TSP patients present with significant lymphocytic perivascular cuffing comprising numerous CD4$^+$ and CD8$^+$ cells (38,39). Similarly, a highly increased number of CD8 HTLV-I-specific CTL can also be measured in the cerebrospinal fluid (CSF) of persons with HAM/TSP, compared to asymptomatic carriers or to persons with other HTLV-I-associated diseases (40,41,42). Three putative pathways of T-cell contribution to neuropathogenesis can be hypothesized, which might overlap, complement and interact mutually. The first one is a direct toxic effect of CD8$^+$ cells with a hyperactive and deregulated CTL response in the brain that primarily attacks HTLV-I-infected cells but also induces bystander damage of the surrounding CNS tissue (43,44,45). The second mechanism is the synthesis of viral Tax protein, a potent viral transcriptional activator, by infected T lymphocytes and/or astrocytes. Tax is capable of inducing an array of host cellular genes as potential mediators of neuronal damage.

Thus Tax-induced perturbation of the astrocytic protein pattern might affect neuronal survival as astrocytes have a crucial role in CNS homeostasis. The third mechanism is an alteration of the cytokine spectrum in the CNS of patients with HAM/TSP either as a result of direct viral infection of T-lymphocytes or as an indirect consequence of secreted viral Tax protein produced by infected cells.

Resident CNS cells such as microglia and astroglia are additional, and on some occasions major, cellular sources of T-cell cytokines in CNS diseases. Altered cytokine production has been described in the CNS and in the periphery of HAM/TSP patients. Increased levels of IFN-γ, TNF-α and IL-6 are measured in the serum and in the CSF (46); furthermore the mRNA for IL-1β, IL-2, TNF-α and IFN-γ is upregulated in peripheral blood lymphocytes of HAM/TSP patients compared with asymptomatic carriers or seronegative donors (42). A high frequency of HTLV-I-specific CD4+ T cells expressing IFN-, TNF-, and IL-2 are present in HAM/TSP patients compared to asymptomatic carriers with similarly high proviral loads (47).

It is hypothesized for TNF-α, IFN-γ, IL-2 and IL-6 that these cytokines contribute to the pathogenesis of inflammatory HTLV-associated CNS disease. In astrocytes the production of TNF-α is stimulated by secreted viral Tax protein, but also by direct contact with infiltrating T-cells. The discovery of numerous TNF-α$^+$ CD4$^+$ T-cells in patients with inflammatory HTLV-induced lesions, but not in asymptomatic carriers supports the hypothesis that bystander damage by TNF-α producing CD4$^+$ T cells is involved in the active lesions of HAM/TSP (47). Similarly, TNF-α is expressed by perivascular infiltrating macrophages, astrocytes and microglia in patients with active-chronic inflammatory HAM/TSP lesions (48). The role of TNF-α might include stimulation of a chronic inflammatory reaction as it induces overexpression of MHC class I and synthesis of other proinflammatory cytokines. In addition, it reduces the microglial glutamate uptake with subsequent oligodendrocyte damage and neurotoxicity by enhanced glutamate concentrations (49).

The cytokine TNF-α but also IL-1ß and neurotransmitters are the most important stimulators of IL-6 production by astrocytes and microglia within the CNS (50). In HAM/TSP patients increased levels of IL-6 may also be due to secretion by microglia that upregulate its production after infection with HTLV-I or incubation with Tax protein (51,52,53). Other sources of IL-6 in the brain of HAM/TSP patients are astrocytes which are stimulated by contact with infected T-cells, and brain-specific endothelial cells exposed to Tax protein (49,54). Besides controlling growth and differentiation of immune cells IL-6 is a trophic factor that can support neuronal and glial differentiation and survival (55,56). In addition to these potentially beneficial effects, there is growing awareness of the destructive potential of IL-6 in the brain (57). Indirect evidence suggests that elevated IL-6 potentiates neural injury in neurodegenerative diseases, possibly by stimulation of nitric oxide production and consequently induction of free radical–induced tissue damage (58).

Similar mechanisms might also play a role in the HTLV-infected brain.

According to the bystander damage hypothesis, IFN-γ may also participate in the induction of nonspecific cell death and inflammation in HAM/TSP patients (59,60). CD8$^+$ cells in HAM/TSP patients produce IFN-γ in recognition of HTLV-I antigens bound to HLAs on the infected CD4$^+$ T-cells (47), and also activated HTLV-specific T-helper cells can produce IFN-γ which then augments CTL effector functions (47). The IFN-γ concentrations in CSF and serum were significantly elevated in patients with HAM/TSP compared to the patients with other neurological diseases and to HTLV-I-seropositive carriers. (61). IFN-γ was also detected in perivascular macrophages, astrocytes and microglia in active-chronic inflammatory lesions of HAM/TSP patients (48). Similar to TNF-α, IFN-γ plays an important role in many inflammatory processes and harbors both neuroprotective and neurotoxic properties (62). For Multiple Sclerosis IFN-γ has been accused to contribute to or even accelerate demyelination as well as to delay the remyelination process by a toxic effect on oligodendrocytes (63). The participation in the pathogenesis of HAM/TSP might involve a similar mechanism. A third cytokine that may contribute to the ongoing state of CNS and PNS inflammation in the HAM/TSP patients is IL-2 which is upregulated in HAM/TSP patients by viral Tax (64,65). IL-2 has an important role in lymphocyte proliferation and in arming effector T-cells, including both T-helper cells and CTLs. IL-2 has also recently been shown to rescue secondary expansion and function in CTLs depleted of CD4$^+$ T-cell help. Increased levels of IL-2 and IL-2R leads to enhanced polyclonal T-cell proliferation in HAM/TSP patients (42) and thus might start a process of T-cell-induced inflammatory CNS damage in these patients (66).

### 3.2. Lentivirinae

Infections of man and various animals by lentiviruses do not only result in peripheral symptoms but may also induce neurological lesions and severe symptoms. This is well established for infections with Visna virus (VV) which infects sheep, caprine arthritis/encephalitis virus (CAEV) in goats, SIV in monkeys, FIV in cats and HIV in humans. Although VV and CAEV do not cause immunodeficiency, they share several features pertinent to the establishment of neuropathologic lesions with those that induce immunodeficiency (67). All lentiviruses infect the CNS hematogenously via infected monocytes and/or lymphocytes which transport their infectious burden as "Trojan horses" into the brain. Free virus may also enter the CNS, either directly or through infection of endothelial cells. The main target for all lentiviruses in the CNS are cells of the monocyte/macrophage/microglial lineage. The discrepancy between the frequency of productively infected cells and the extent and character of pathological lesions indicates that a mechanism other than the direct effect of the virus contributes to the evolution of CNS lesions (68).

### 3.2.1. Feline immunodeficiency virus

FIV-infected cats have become a non-rodent model of AIDS because of the similarity of the virus with

HIV. Neurological dysfunctions occur in 20-40% of naturally infected cats and include hind limb paralysis, delayed reflexes, irritability and disorientation (69). The precise mechanism leading to neurological manifestations is unknown. Since no infected neurons were found and since there is no correlation between viral load and progression of CNS alterations, it is hypothesized that infection of microglia and infiltrating macrophages results in an increased immune activation state and in the release of potentially neurotoxic factors.

Microglia are infected by FIV very early after peripheral entry of the virus, and represent a persistent brain-resident viral reservoir (70). Astrocytes are a further target for FIV-infection (71).

Microglia are supposed to be main players of FIV-associated immunopathogenesis. Concomitant with the onset of viral reproduction, infected microglia undergo an activation process, characterized by the enhanced expression of MHC molecules and neurotoxic factors (70). Similar to the retroviruses described above those factors might include dysregulated cytokine levels. TNF-α is enhanced in the CNS of FIV-infected cats with microglia and, to a lesser extent, astrocytes as the main producer (72); the stimulation is at least partly a consequence of intracellular envelope expression (73). This increase in TNF-α expression is hypothesized to be involved in the early stages of FIV-induced encephalitis in cats and to contribute to neurotoxicity (68). The expression of other cytokines such as IL-1β is also modulated and might represent another pathogenetic factor (73).

### 3.2.2. Human and simian immunodeficiency virus

Striking similarities in the neuropathogenesis of SIV and HIV are evident; therefore these two viruses are discussed here together with emphasis on HIV.

Infection of the CNS is detected in virtually all HIV-positive individuals (10); neurological symptoms include meningitis, peripheral neuropathies and AIDS dementia complex (ADC) with cognitive, motor and behavioural dysfunctions (74) As neuropathological correlates atrophy, multinucleated giant cells, reactive astrogliosis, microgliosis, loss of neurons and inflammatory infiltrates are found (75).
Both SIV and HIV mainly infect microglia and perivascular macrophages; astrocytes are also viral targets, but to a lesser extent (10). Since HIV and SIV do not infect neurons indirect mechanisms of neurodegeneration have been proposed. As in the case of other retroviruses, growing evidence suggests a central role for immune elements in the neuropathogenesis of SIV- and HIV-infection.

HIV-infected infiltrating macrophages and T-cells can act as "Trojan horses" and transport the virus into the brain, presumably already during primary viraemia (76,77). Thus these immune cells prepare the basics for the virus-induced neurological dysfunction of HIV-infected patients. Furthermore parenchymal microglia, macrophages and T-cells are productively infected by HIV-1, HIV-2 or SIV and thus generate the viral burden in the brain and represent viral reservoirs (78,79,80). The infection of astrocytes establishes another (lifelong) viral reservoir in the CNS. Although the replication cycle in astrocytes does not or only marginally end in the production of infectious viral particles (10), infected astrocytes produce and secrete high amounts of regulatory viral proteins like Tat and Nef. Together with the viral envelope proteins gp120 and gp41 derived from infected microglia these proteins are discussed to be neurotoxic, to contribute to the increased oxidative stress and thus to mediate HIV-associated brain damage (81).

Early after cerebral infection by HIV or SIV a broad microglial activation can be detected that increases with the course of the infection and runs parallel to viral replication (83,83). This microglial activation initiates a series of neuroprotective processes including upregulation of glutamate transporters and scavenger molecules that allow an efficient removal of harmful excitotoxins and dead cells. Furthermore the production of nerve growth factor, neurotrophins and cytokines is augmented upon activation and supports the regeneration of damaged neurons or prevents from inflammation-induced brain damage (84,2). On the other hand activated microglia are hypothesized to contribute to neurodegeneration and neurological symptoms of HIV-dementia patients (85). In order to fulfil their functions in the cerebral innate immune response microglia produce a variety of effector molecules (excitatory amino acids, proteases, end products of oxidative stress, oxide radicals, cytokines) many of which are potentially harmful for the exquisitely vulnerable neurons (86,87). Increased levels of excitotoxins and end products of oxidative stress were found in the CSF of patients with AIDS dementia complex (88). Similarly, microglia-derived cytokines and chemokines display both neurotoxic effects as described further below (2).

Astrocytes as an integral part of the blood-brain barrier get early in direct touch with virus and/or with infiltrating HIV-infected blood cells and thus are supposed to be infected early in the course of cerebral infection. The exposition of astrocytes to gp120 or Tat stimulates the synthesis of adhesion molecules and thus targets further leukocytes to the CNS (89,90). Putative consequences are a facilitated infiltration of HIV-infected cells but also a more efficient antiviral immune response (89). HIV counteracts this increased antiviral defence by inducing production and secretion of the viral regulator protein Nef. Secreted Nef protein affects the antigen presentation of infected and neighbouring astrocytes by downmodulation of MHC I surface expression. This mechanism protects infected cells from CTL killing and thus favours viral spreading (91,92).

After cerebral infection by SIV or HIV astrocytes react with a dramatic increase in complement synthesis, mediated either directly by the virus or indirectly by inflammatory cytokines (93,94,95). Via this complement production astrocytes provide the first line of cerebral immune defence with lysis of virions and infected cells, cell activation and attraction of microglia, peripheral T-cells and macrophages as main complement functions.

The HIV virions can initiate the complement activation cascade by all three pathways, either antibody-dependently or, in the absence of antibodies, by direct interaction of the viral envelope proteins gp41 and gp120 with the starter molecules of the complement cascade (96). After cerebral infection HIV-induced increase in complement synthesis and complement activation can influence and interact with numerous immunological and virological processes. Complement can participate in the limitation of viral load by direct lysis of invading pathogens or infected cells. Furthermore, opsonization of HIV with complement fragments might support the phagocytosis by microglia or astrocytes. However, virions opsonized with complement fragment iC3b might, in analogy to the situation in the periphery (97), interact with complement receptor-positive cells like microglia or astrocytes and consequently infect them with higher efficacy.

The anaphylatoxins C3a and C5a, cleavage products of complement factors C3 and C5, exert various biological functions in the brain (11). These include attraction of peripheral immune cells, chemotactic activity towards microglia and astrocytes, cell activation and induction of signalling pathways, modulation of cytokine expression and induction of apoptosis, and cell cycle progression. The sensitivity of cells against C5a is increased in the HIV-infected brain since the viral Nef proteins alters the synthesis of CD88, the receptor for C5a (98).

Several hints argue for a potential role of chronically activated complement as a detrimental mediator of HIV-induced neuropathogenesis. As described in the introduction complement has been associated to a variety of neurodegenerative conditions like Alzheimer's disease, Huntington's disease, multiple sclerosis and stroke (11). The viral envelope protein gp41 down-modulates the expression of the membrane-bound negative complement regulator CD59 on neurons and thus further sensitizes them against complement-dependent bystander lysis (99). Furthermore HIV-induced complement activation results in generation of C5a that was reported to induce apoptosis of neuronal cells (100).

For all these functions sufficient levels of complement within the brain are a prerequisite. Whereas the spontaneous constitutive expression of complement is low in the CNS, the synthesis can be markedly upregulated by inflammatory cytokines (11). Furthermore, viral infection by HIV directly upregulates the expression of complement factors in astrocytes and neurons by stimulation of the corresponding promoter (93,94,101). These results fit well to the increased levels of C3 found in the CSF of HIV-infected patients with neurological symptoms *in vivo* compared to CSF from uninfected individuals (102). Furthermore, recent own immunohistochemical analyses revealed significant complement production in the brain of HIV-infected patients compared with uninfected control tissue (95).

Both pro-inflammatory and anti-inflammatory cytokines and chemokines are modulated in their expression in the HIV-infected brain either by the immunological interaction between virus and host or by the release of viral proteins (2). The subsequent imbalance of cytokine and chemokine levels contributes to neuropathologic manifestations of the infection such as microgliosis, astrocytosis and neuronal dysfunction or death. Some cytokines show a strict correlation between enhanced CSF concentrations and the symptoms of dementia (2). On the other hand upregulation of some cytokines might contribute to neuroprotection and limit viral spreading and virus-induced damage. Putative mechanisms for both aspects are discussed in the following.

Cytokines/chemokines are central regulators for the infiltration of blood-derived immune cells through the blood-brain barrier with improved antiviral defence and/or enhanced cerebral infiltration of virus-producing cells as putative consequences. Deficits in lymphocyte recruitment and/or trafficking into the brain are discussed to be associated with high viral load and onset of HIV-associated dementia (103).

Cytokines/chemokines orchestrate the immune defence with either activation (IFN-γ, IFN-α, TNF-α) or inactivation (IL-4, IL-10) of astrocytes and microglia, T-cells and monocytes. HIV-infection of the brain results in upregulation of cytokines from both groups and thus shifts the sensitive balance in the CNS (2). Cytokine-driven cell activation and excessive inflammation might be a central mechanism of brain damage in the HIV-infected brain; in addition, cytokine-induced inhibition of normal cell functions or induction of apoptosis might play a role in the development of HIV neuropathogenesis (10,104).

Cytokines modulate HIV transcription and replication in brain cells. IL-4 and IL-10 enhance the replication of HIV in microglia, and tumor growth factor-beta (TGF-β)-associated signalling proteins change the activity of the viral long terminal repeats in astrocytes (105,106). Other reports describe the induction of HIV replication in CNS cell cultures by pro-inflammatory IFN-γ, IL-1β and TNF-α (107). Since the cerebral expression of these cytokines is enhanced after HIV-infection, there seems to be a vicious circle for the mutual stimulation of viral replication and cytokine synthesis.

Cytokines/chemokines still have an additional possibility to modulate viral spreading after cerebral infection by SIV or HIV. Inflammatory cytokines regulate the expression of the chemokine receptor CXCR4 in astrocytes which represents an essential co-receptor for HIV attachment to the host cell (108). Similarly the synthesis of CCR5, the other co-receptor for HIV infection, was enhanced in microglia under the influence of cytokines (105). Thus cytokines can directly determine the susceptibility of different brain cells towards the infection by HIV. Furthermore, since binding of HIV envelope protein gp120 to chemokine receptors elicits intracellular signalling and thus acts as chemokine agonist in astrocytes, the modulation of receptor expression also exerts an influence on the activation state of the cells (19,110). The susceptibility of brain cells towards infection can also be

influenced directly by the levels of certain chemokines which represent the natural ligands for the HIV co-receptors. The direct competition between the viral envelope protein gp120 and chemokines for receptor binding underlines the significance of chemokine levels in the brain (111). One example is the chemokine stromal cell-derived factor-1 (SDF-1 ), the ligand of CXCR4. The upregulation of SDF-1 as found in the brain of HIV-infected patients with encephalitis might help to protect brain cells from infection by shifting the competition between binding of chemokine and gp120 in favour of SDF-1 alpha (112).

The cytokines TNF-$\alpha$, IL-1$\beta$ and IL-6, which are upregulated in the CSF after HIV-infection, are potent inducers of complement factor C3 expression in astrocytes (2,113). Thus these inflammatory cytokines might contribute to the complement effects in the CNS, either beneficial or detrimental. TNF-$\alpha$ also enhances the production of negative regulators of complement activation like factor H and C1-inhibitor (114) and thus provides an effective counterbalance for the induction of complement factors. Furthermore, anti-inflammatory cytokines such as IL-10 that are increased in astrocytes by HIV or its envelope proteins suppress an excessive immune reaction and immune-mediated injury of the CNS (115). Besides, IL-10 induces the secretion of nerve growth factor and can thus provide a neurotrophic support to injured neurons in the HIV-infected brain (116). However, the price for that anti-inflammatory protection and counteraction of an excessive inflammation is high and includes disturbance of antiviral defence in the CNS and favouring of viral spreading, since increased IL-10 levels suppress efficient microglial proliferation and antigen presentation (117), inhibit an effective T helper cell response to antigens (118) and impair monocyte/macrophage functions (119).

## 4. PERSPECTIVES

The multifaceted interactions between the cerebral innate immunity and invading retroviruses are supposed to influence the retrovirus-induced neuropathogenesis in a both protective and detrimental manner. Since the different immune players in the CNS do not act separately but form a complex functional immunity network with each other and with infiltrating antiviral weapons from the periphery it is difficult to weight the net effect in an overall picture. Potent anti-retroviral defence and immune cell activation has to be weighted against harmful inflammation within the sensitive brain tissue; anti-inflammatory protection should be regarded versus favour of viral replication and spreading; attraction of efficient peripheral immune cells has to be balanced against infiltration of infected "Trojan horses". Thus a global view of the retrovirus-infected CNS can provide interesting insights into a very particular immune constellation and help to develop immune-based therapeutic approaches to limit infectious neuropathogenesis.

## 5. ACKNOWLEDGEMENTS

## 6. REFERENCES

1. F. Aloisi, E. Ambrosini, S.Columba-Cabezas, R. Magliozzi and B.Serafini: Intracerebral regulation of immune responses. *Ann. Med.* 33, 510-515 (2001)
2. C. Speth, M.P. Dierich and S. Sopper: HIV-infection of the central nervous system: the tightrope walk of innate immunity. *Mol Immunol.* 422), 213-28 (2005)
3. G. Raivich, M, Bohatschek, C.U. Kloss, A. Werner, L.L. Jones and G.W. Kreutzberg: Neuroglial activation repertoire in the injured brain: graded response, molecular mechanisms and cues to physiological function. *Brain Res Brain Res Rev.* 30, 77-105 (1999)
4. F. Aloisi, G. Penna, J.Cerase, B.M. Iglesias, and L. Adorini: IL-12 production by central nervous system microglia is inhibited by astrocytes. *J. Immunol.,* 159, 1604-1612 (1997)
5. H. Neumann, J. Boucraut, C. Hahnel, T. Misgeld and H. Wekerle: Neuronal control of MHC class II inducibility in rat astrocytes and microglia. *Eur. J. Neurosci.* 8, 2582-2590 (1996)
6. C. Eder, R. Klee and U. Heinemann: Distinct soluble astrocytic factors induce expression of outward $K^+$ currents and ramification of brain macrophages. *Neurosci. Lett.* 226, 147-150 (1997)
7. E.N. Benveniste, V.T. Nguyen and D.R. Wesemann: Molecular regulation of CD40 gene expression in macrophages and microglia. *Brain Behav. Immun.* 18, 7-12 (2004)
8. S. Nataf, P.F. Stahel, N. Davoust, S.R. Barnum: Complement anaphylatoxin receptors on neurons: new tricks for old receptors? *Trends Neurosci.* 22, 397-402 (1999)
9. S. Rivest: Molecular insights on the cerebral innate immune system. *Brain. Behav. Immun.* 17, 13-19 (2003)
10. R. Brack-Werner: Astrocytes: HIV cellular reservoirs and important participants in neuropathogenesis. *AIDS*, 13:1-22 (1999)
11. C. Speth, M.P. Dierich and P. Gasque: Neuroinvasion by pathogens: a key role of the complement system. *Mol. Immunol.* 38, 669-679 (2002)
12. Y. Dong and E.N. Benviste: Immune function of astrocytes. *Glia* 36:180-190 (2001)
13. N.P. Hailer, B. Glomsda and R.A. Blaheta: Astrocytic factors down-regulate the expression of major histocompatibility complex-class-II and intercellular adhesion molecule-1 on human monocytes. *Neurosci Lett.* 298, 33-36 (2001)
14. V.W. Yong, T. Tejada-Berges, C.G. Goodyer, J.P. Antel and F.P. Yong: Differential proliferative response of human and mouse astrocytes to gamma-interferon.*Glia* 6, 269-80 (1992)
15. A. Cornet, E. Bettelli, M. Oukka, C. Cambouris, V. Avellana-Adalid, K. Kosmatopoulos and R.S. Liblau: Role of astrocytes in antigen presentation and naive T-cell activation. *J. Neuroimmunol.*106, 69-77 (2000)
16. T.Magnus, A.Chan, R.A. Linker, K.V. Toyka and R. Gold: Astrocytes are less efficient in the removal of apoptotic lymphocytes than microglia cells: implications

for the role of glial cells in the inflamed central nervous system. *J Neuropathol Exp Neurol.* 61, 760-766 (2002)

17. C. Speth, R. Würzner, H. Stoiber, M.P. Dierich: The complement system: pathophysiology and clinical relevance. *Wien. Klien. Wochenschr* 11110), 378-391 (1999)

18. S.K. Singhrao, J.W. Neal, N.K. Rushmere, B.P. Morgan and P. Gasque: Spontaneous classical pathway activation and deficiency of membrane regulators render human neurons susceptible to complement lysis. *Am J Pathol.* 157, 905-918 (2000)

19. P. Gasque, A. Ischenko, J. Legoedec, C. Mauger, M.T. Schouft and M. Fontaine: Expression of the complement classical pathway by human glioma in culture. *J. Bio. Chem.* 268, 25068-25074 (1993)

20. H.G. Rus, L.M. Kim, F.I. Niculescu and M.L. Shin: Induction of C3 expression in astrocytes is regulated by cytokines and Newcastle Disease Virus. *J. Immunol.* 148, 928-933 (1992)

21. R.J. Miller and O. Meucci,: AIDS and the brain: is there a chemokine connection? *Trends Neurosci.* 22, 471-479 (1999)

22. Y. Sei, P.K. Arora, P. Skolnick and I.A. Paul: Spatial learning impairment in a murine model of AIDS. *FASEB J* 611), 3008-3013 (1992)

23. V.J. Sanders, C.A. Wiley and R.L. Hamilton: The mechanisms of neuronal damage in retroviral infections of the nervous system. *Curr. Top. Microbiol. Immunol.* 253, 179-201 (2001)

24. S. Askovic, C. Favara, F.J. McAtee and J.L. Portis: Increased expression of MIP-1 alpha and MIP-1 beta mRNAs in the brain correlates spatially and temporally with the spongiform neurodegeneration induced by a murine oncornavirus. *J. Virol.* 756), 2665-2674 (2001)

25. W. Choe, G. Stoica, W. Lynn and P.K. Wong: Neurodegeneration induced by MoMuLV-ts1 and increased expression of Fas and TNF-alpha in the central nervous system. *Brain Res.* 779 (1-2), 1-8 (1998)

26. W. Qiang, J.M. Cahill, J. Liu, X. Kuang, N. Liu, V.L. Scofield, J.R. Voorhees, A.J. Reid, M. Yan, W.S. Lynn and P.K. Wong: Activation of transcription factor Nrf-2 and its downstream targets in response to moloney murine leukemia virus ts1-induced thiol depletion and oxidative stress in astrocytes. *J. Virol.* 7821), 11926-38 (2004)

27. J.F. Zachary, T.V. Baszler, R.A. French and K.W. Kelley: Mouse Moloney leukemia virus infects microglia but not neurons even though it induces motor neuron disease. *Mol. Psychiatry* 22), 104-106 (1997)

28. T.V. Baszler and J.F. Zachary: Murine retroviral neurovirulence correlates with an enhanced ability of virus to infect selectively, replicate in, and activate resident microglial cells. Am. *J. Pathol.* 1383), 655-671 (1991)

29. R.M. Nagra, M.P. Heyes and C.A. Wiley: Viral load and its relationship to quinolinic acid, TNF alpha, and IL-6 levels in the CNS of retroviral infected mice. *Mol. Chem. Neuropathol.* 223), 143-160 (1994)

30. M. Pickering, D. Cumiskey and J.J. O'Connor: Actions of TNF-alpha on glutamatergic synaptic transmission in the central nervous system. *Exp. Physiol.* 905), 663-70 (2005)

31. R. N. Saha and K. Pahan: Tumor necrosis factor-alpha at the crossroads of neuronal life and death during HIV-

associated dementia. J. Neurochem. 865), 1057-1071 (2003)

32. R. Nakai, S. Takase-Yoden and R. Watanabe: Analysis of the distribution of neuropathogenic retroviral antigens following PVC211 or A8-V infection. Microbiol. Immunol. 4912), 1075-1081 (2005)

33. M. Czub, S. Czub, M. Rappold, S. Mazgareanu, S. Schwender, M. Demuth, A. Hein and R. Dorries: Murine leukemia virus-induced neurodegeneration of rats: enhancement of neuropathogenicity correlates with enhanced viral tropism for macrophages, microglia, and brain vascular cells. *Virology* 2141), 239-244 (1995)

34. T. Uchiyama: Human T cell leukemia virus type I (HTLV-I. and human diseases. *Ann. Rev. Immunol.* 15, 15-37 (1997.

35. V. Zaninovic: Possible etiologies for tropical spastic paraparesis and human T lymphotropic virus I-associated myelopathy. *Braz. J. Med Biol. Res.* 371), 1-12 (2004.

36. J.H. Richardson, A.J. Edwards, J.K. Cruickshank, P. Rudge and A.G. Dalgleish: In vivo cellular tropism of human T-cell leukemia virus type 1. *J. Virol.* 6411), 5682-5687 (1990)

37. C. Grant, K. Barmak, T. Alefantis, J. Yao, S. Jacobson and B. Wigdahl: Human T cell leukaemia virus type I and neurologic disease: events in bone marrow, peripheral blood, and central nervous system during normal immune surveillance and neuroinflammation. *J. Cell. Physiol.* 190: 133-159 (2002.

38. J. Casseb and A.C. Penalva-de-Oliveira: The pathogenesis of tropical spastic paraparesis/human T-cell leukemia type I-associated myelopathy. *Braz. J. Med Biol. Res.* 3312), 1395-1401 (2001.

39. F. Umehara, S. Izumo, M. Nakagawa, A.T. Ronquillo, K. Takahashi, K. Matsumuro, E. Sato and M. Osame: Immunocytochemical analysis of the cellular infiltrate in the spinal cord lesions in HTLV-I-associated myelopathy. *J. Neuropathol. Exp. Neurol.* 524), 424-430 (1993)

40. S. Jacobson, H. Shida, D.E. McFarlin, A.S. Fauci and S. Koenig: Circulating CD8+ cytotoxic T lymphocytes specific for HTLV-I pX in patients with HTLV-I associated neurological disease. *Nature* 3486298), 245-248 (1990)

41. M. Nagai, R. Kubota, T.F. Greten TF, J.P. Schneck, T.P. Leist and S. Jacobson: Increased activated human T cell lymphotropic virus type I (HTLV-I. Tax11-19-specific memory and effector CD8+ cells in patients with HTLV-I-associated myelopathy/tropical spastic paraparesis: correlation with HTLV-I provirus load. *J Infect Dis.* 1832), 197-205 (2001)

42. S. Jacobson Immunopathogenesis of human T cell lymphotropic virus type I-associated neurologic disease. *J. Inf. Dis.* 186 Suppl 2, S187-92 (2002)

43. K. Barmak, E.W. Harhaj and B. Wigdahl: Mediators of central nervous system damage during the progression of human T-cell leukemia type I-associated myelopathy/tropical spastic paraparesis. *J. Neurovirol.* 95), 522-529 (2003)

44. T.J. Lehky, C.H. Fox, S. Koenig, M.C. Levin, N. Flerlage, S. Izumo, E. Sato, C.S. Raine, M. Osame and S. Jacobson: Detection of human T-lymphotropic virus type I (HTLV-I. tax RNA in the central nervous system of HTLV-I-associated myelopathy/tropical spastic paraparesis

patients by in situ hybridization. *Ann. Neurol.* 372), 167-175 (1995)

45. H. Hara, M. Morita, T. Iwaki, T. Hatae, Y. Itoyama, T. Kitamoto, S. Akizuki, I. Goto and T. Watanabe: Detection of human T lymphotrophic virus type I (HTLV-I. proviral DNA and analysis of T cell receptor V beta CDR3 sequences in spinal cord lesions of HTLV-I-associated myelopathy/tropical spastic paraparesis. J. Exp. Med. 1803), 831-839 (1994)

46. Y. Kuroda and M. Matsui: Cerebrospinal fluid interferon-gamma is increased in HTLV-I-associated myelopathy. J. Neuroimmunol. 422), 223-226 (1993)

47. P.K. Goon, T. Igakura, E. Hanon, A.J. Mosley, B. Asquith, K.G. Gould, G.P. Taylor, J.N. Weber and C.R. Bangham: High circulating frequencies of tumor necrosis factor alpha- and interleukin-2-secreting human T-lymphotropic virus type 1 (HTLV-I)-specific CD4+ T cells in patients with HTLV-I-associated neurological disease. J. Virol 7717), 9716-9722 (2003)

48. F. Umehara, S. Izumo, A.T. Ronquillo, K. Matsumuro, E. Sato and O.M. Osame: Cytokine expression in the spinal cord lesions in HTLV-I-associated myelopathy. J. Neuropathol Exp. Neurol 531), 72-77 (1994)

49. R. Szymocha, H. Akaoka, C. Brisson, P. Beurton-Marduel, A. Chalon, A. Bernard, M. Didier-Bazes, M.F. Belin and P. Giraudon: Astrocytic alterations induced by HTLV type 1-infected T lymphocytes: a role for Tax-1 and tumor necrosis factor alpha. AIDS Res. Hum. Retrovir. 1616), 1723-1729 (2000)

50. D.L. Gruol and T.E. Nelson. Physiological and pathological roles of interleukin-6 in the central nervous system. *Mol. Neurobiol.* 153), 307-339 (1997)

51. S. Dhib-Jalbut, P.M. Hoffman, T. Yamabe, D. Sun, J. Xia, H. Eisenberg, G. Bergey and F.W. Ruscetti: Extracellular human T-cell lymphotropic virus type I Tax protein induces cytokine production in adult human microglial cells. *Ann. Neurol.* 365), 787-790 (1994)

52. R.J. Fox, M.C. Levin and S. Jacobson: Tumor necrosis factor alpha expression in the spinal cord of human T-cell lymphotrophic virus type I associated myelopathy/tropical spastic paraparesis patients. *J. Neurovirol.* 25), 323-329 (1996)

53. M. Nagai, S. Ijichi, W.W. Hall and M. Osame: Differential effect of TGF-beta 1 on the in vitro activation of HTLV-I and the proliferative response of CD8+ T lymphocytes in patients with HTLV-I-associated myelopathy (HAM/TSP). *Clin. Immunol. Immunopathol.* 773), 324-331 (1995)

54. O. Rott, U. Tontsch, B. Fleischer and E. Cash: Interleukin-6 production in "normal" and HTLV-I tax-expressing brain-specific endothelial cells. *Eur. J. Immunol.* 238), 1987-1991 (1993)

55. K. Frei, U.V. Malipiero, T.P. Leist, R.M. Zinkernagel, M.E. Schwab and A. Fontana: On the cellular source and function of interleukin 6 produced in the central nervous system in viral diseases. *Eur. J. Immunol.* 194), 689-694 (1989)

56. D.E. Brenneman, M. Schultzberg, T. Bartfai and I. Gozes: Cytokine regulation of neuronal survival. *J. Neurochem.* 582), 454-460 (1992)

57. A.I. Kaplin, D.M. Deshpande, E. Scott, C. Krishnan, J.S. Carmen, I. Shats, T. Martinez, J. Drummond, S. Dike, M. Pletnikov, S.C. Keswani, T.H. Moran, C.A. Pardo, P.A. Calabresi and D.A. Kerr: IL-6 induces regionally selective spinal cord injury in patients with the neuroinflammatory disorder transverse myelitis. *J. Clin. Invest.* 11510), 2731-2741 (2005)

58. X. Yu and R.H. Kennedy, S.J. Liu: JAK2/STAT3, not ERK1/2, mediates interleukin-6-induced activation of inducible nitric-oxide synthase and decrease in contractility of adult ventricular myocytes. *J Biol Chem.* 27818), 16304-16309 (2003)

59. S. Daenke and C.R. Bangham: Do T cells cause HTLV-I-associated disease?: a taxing problem. *Clin. Exp. Immunol.* 962), 179-181 (1994)

60. S. Ijichi, S. Izumo, N. Eiraku, K. Machigashira, R. Kubota, M. Nagai, N. Ikegami, N. Kashio, F. Umehara and I. Maruyama: An autoaggressive process against bystander tissues in HTLV-I-infected individuals: a possible pathomechanism of HAM/TSP. *Medical Hypotheses* 416), 542-547 (1993)

61. T. Furuya, T. Nakamura, T. Fujimoto, S. Nakane, C. Kambara, S. Shirabe, S. Hamasaki, M. Motomura and K. Eguchi: Elevated levels of interleukin-12 and interferon-gamma in patients with human T lymphotropic virus type I-associated myelopathy. *J. Neuroimmunol.* 951-2), 185-189 (1999)

62. M. Schroeter and S. Jander: T-cell cytokines in injury-induced neural damage and repair. *Neuromol Medicine* 73), 183-195 (2005)

63. P. Mana, D. Linares, S. Fordham, M. Staykova and D. Willenborg: Deleterious Role of IFN{gamma} in a Toxic Model of Central Nervous System Demyelination. *Am. J. Pathol.* 1685), 1464-1473 (2006)

64. F. Bex and R.B. Gaynor: Regulation of gene expression by HTLV-I Tax protein. *Methods* 161), 83-94 (1998)

65. S.J. Greenberg: Human retroviruses and demyelinating diseases. *Neurol. Clin.* 131), 75-97 (1995)

66. J. Casseb and A.C. Penalva-de-Oliveira: The pathogenesis of tropical spastic paraparesis/human T-cell leukemia type I-associated myelopathy. *Braz J Med Biol Res.* 3312), 1395-401 (2000)

67. G. Georgsson: Neuropathologic aspects of lentiviral infections. *Ann. N. Y. Acad. Sci.* 724, 50-67 (1994)

68. K.E. Peterson and B. Chesebro: Influence of proinflammatory cytokines and chemokines on the neuropathogenesis of oncornavirus and immunosuppressive lentivirus infections. *Curr. Top. Microbiol. Immunol.* 303, 67-95 (2006)

69. T.R. Phillips, O. Prospero-Garcia, D.W. Wheeler, P.C. Wagaman, D.L. Lerner, H.S. Fox, L.R. Whalen, F.E. Bloom, J.H. Elder and S.J. Henriksen: Neurologic dysfunctions caused by a molecular clone of feline immunodeficiency virus, FIV-PPR. *J Neurovirol.* 26), 388-396 (1996)

70. A. Hein, J.P. Martin and R. Dorries: Early pathological changes in the central nervous system of acutely feline-immunodeficiency-virus-infected cats. *Virology* 3432), 162-170 (2005.

71. K. Nakagaki, K.Nakagaki, K. Takahashi,, D. Schols, E. De Clercq E and T. Tabira: CXCR4 is the primary receptor for feline immunodeficiency virus in astrocytes. *J. Neurovirol.* 75), 487-492 (2001)

72. A. Poli, M. Pistello, M.A. Carli, F. Abramo, G. Mancuso, E. Nicoletti and M. Bendinelli: Tumor necrosis factor-alpha and virus expression in the central nervous system of cats infected with feline immunodeficiency virus. *J Neurovirol* 55), 465-473 (1999)

73. F. Noorbakhsh, Q. Tang, S. Liu, C. Silva, G. van Marle and C. Power: Lentivirus envelope protein exerts differential neuropathogenic effects depending on the site of expression and target cell. *Virology* 3482), 260-276 (2006)

74. F. Gray, F. Scaravilli, I. Everall, F. Chretien, S. An, D. Boche, H. Adle-Biassette, L. Wingertsmann, M. Durigon, B. Hurtrel, F. Chiodi, J. Bell, and P. Lantos: Neuropathology of early HIV-1 infection. *Brain Pathol*. 6, 1-15 (1996)

75. H. Budka: Neuropathology of human immunodeficiency virus infection. *Brain Pathol* 1, 163-175 (1991)

76. L. E. Davis, B. L. Hjelle, V. E. Miller, D. L. Palmer, A. L. Llewellyn, T. L. Merlin, S. A. Young, R. G. Mills, W. Wachsman and C. A. Wiley: Early viral brain invasion in iatrogenic human immunodeficiency virus infection. *Neurology* 42, 1736-1739 (1992)

77. M. Demuth, S. Czub, U. Sauer, E. Koutsilieri, P. Haaft, J. Heeney, C. Stahl-Hennig, V. ter Meulen, and S. Sopper: Relationship between viral load in blood, cerebrospinal fluid, brain tissue and isolated microglia with neurological disease in macaques infected with different strains of SIV. *J Neurovirol* 6, 187-201 (2000)

78. M.A. Cosenza, M.L. Zhao, Q. Si and S.C. Lee: Human brain parenchymal microglia express CD14 and CD45 and are productively infected by HIV-1 in HIV-1 encephalitis. *Brain Pathol* 12, 442-455 (2002)

79. S. Sopper, E. Koutsilieri, C. Scheller, S. Czub, P. Riederer and V. ter Meulen: Macaque animal model for HIV-induced neurological disease. *J Neural Transm* 109, 747-766 (2002)

80. E.V. Ryzhova, P. Crino, L. Shawver, S.V. Westmoreland, A.A. Lackner and F. Gonzalez-Scarano: Simian immunodeficiency virus encephalitis: analysis of envelope sequences from individual brain multinucleated giant cells and tissue samples. *Virology* 297, 57-67 (2002)

81. V. Mollace, H.S. Nottet, P. Clayette, M.C. Turco, C. Muscoli, D. Salvemini and C.F. Perno: Oxidative stress and neuroAIDS: triggers, modulators and novel antioxidants. *Trends Neurosci* 24, 411-416 (2001)

82. L. Chakrabarti, M. Hurtrel, M.A. Maire, R. Vazeux, D. Dormont, L. Montagnier and B. Hurtrel: Early viral replication in the brain of SIV-infected rhesus monkeys. *Am J Pathol* 139, 1273-1280 (1991)

83. S. Sopper, M. Demuth, C. Stahl-Hennig, G. Hunsmann, R. Plesker, C. Coulibaly, S. Czub, M. Ceska, E. Koutsilieri, P. Riederer, R. Brinkmann, M. Katz and V. ter Meulen: The effect of simian immunodeficiency virus infection in vitro and in vivo on the cytokine production of isolated microglia and peripheral macrophages from rhesus monkey. *Virology* 220, 320-329 (1996)

84. K. Nakajima, Y. Tohyama, S. Kohsaka, and T. Kurihara: Ability of rat microglia to uptake extracellular glutamate. *Neurosci Lett* 307, 171-174 (2001)

85. M. Kaul, G.A. Garden and S.A. Lipton: Pathways to neuronal injury and apoptosis in HIV-associated dementia. *Nature* 410: 988-994 (2001)

86. E. Koutsilieri, S. Sopper, T. Heinemann, C. Scheller, J. Lan, C. Stahl-Hennig, V. ter Meulen, P. Riederer and M. Gerlach: Involvement of microglia in cerebrospinal fluid glutamate increase in SIV-infected rhesus monkeys (Macaca mulatta). *AIDS Res Hum Retroviruses* 15, 471-477 (1999)

87. M. Schwartz: Macrophages and microglia in central nervous system injury: are they helpful or harmful? *J Cereb Blood Flow Metab* 23: 385-394 (2003)

88. C. Ferrarese, A. Aliprandi, L. Tremolizzo, L. Stanzani, A. De Micheli, A. Dolara and L. Frattola: Increased glutamate in CSF and plasma of patients with HIV dementia. *Neurology* 57, 671-67 (2001)

89. S.E. Woodman, E.N. Benviste, A. Nath and J.W. Berman: Human immunodeficiency virus type 1 TAT protein induces adhesion molecule expression in astrocytes. *J Neurovirol*. 5, 678-84 (1999)

90. S. Toneatto, O. Finco, H. van der Putten, S. Abrignani and P. Annunziata: Evidence of blood-brain barrier alteration and activation in HIV-1 gp120 transgenic mice. *AIDS* 13, 2343-2348 (1999)

91. S.A. Swann, M. Williams, C.M. Story, K.R. Bobbit, R. Fleis and K.L. Collins: HIV-1 Nef blocks transport of MHC class I molecules to the cell surface via a PI 3-kinase-dependent pathway. *Virology* 282, 267-77 (2001)

92. O.O. Yang, P.T. Nguyen, S.A. Kalams, T. Dorfman, H.G. Gottlinger, S. Stewart, I.S. Chen, S. Threlkeld and B.D. Walker: Nef-mediated resistance of human immunodeficiency virus type 1 to antiviral cytotoxic T lymphocytes. *J Virol*. 76, 1626-1631 (2002)

93. C. Speth, G. Stöckl, I. Mohsenipour, R. Würzner, H. Stoiber, C. Lass-Flörl and M.P. Dierich: Human immunodeficiency virus type 1 induced expression of complement factors in human astrocytes. *J Virol;* 75, 2604-2615 (2001)

94. C. Speth, T. Schabetsberger, I. Mohsenipour, G. Stöckl, R. Würzner, H. Stoiber, C. Lass-Flörl and M.P. Dierich. Mechanism of human immunodeficiency virus-induced complement expression in astrocytes and neurons. *J Virol*. 76, 3179-3188 (2002)

95. C. Speth, K. Williams, M. Hagleitner, S. Westmoreland, G. Rambach, I. Mohsenipour, J. Schmitz, R. Wurzner, C. Lass-Florl, H. Stoiber, M.P. Dierich and H. Maier. Complement synthesis and activation in the brain of SIV-infected monkeys. *J Neuroimmunol*. 1511-2), 45-54 (2004)

96. C.Speth, L. Kacani and M.P. Dierich: Complement receptors in HIV infection. *Immunol Rev*. 159, 49-67 (1997)

97. D.C. Montefiori, G. Pantaleo, L.M. Fink, J.T. Zhou, J.Y. Zhou, M. Bilska, G.D. Miralles and A.S. Fauci: Neutralizing and infection-enhancing antibody responses to human immunodeficiency virus type 1 in long-term nonprogressors. *J Infect Dis* 173, 60-67 (1996)

98. B. Kohleisen, E. Shumay, G. Sutter, R. Foerster, R. Brack-Werner, M. Nuesse and V. Erfle: Stable expression of HIV-1 Nef induces changes in growth properties and activation state of human astrocytes. *AIDS* 13, 2331-2341 (1999)

99. Y.H. Chong and M.J. Lee: Expression of complement inhibitor protein CD59 in human neuronal and glial cell

lines treated with HIV-1 gp41 peptides. *J. Neurovirol.* 6, 51-60 (2000)

100. I. Farkas, L. Baranyi, Z.S. Liposits, T. Yamamoto and H. Okada: Complement C5a anaphylatoxin fragment causes apoptosis in TGW neuroblastoma cells. *Neuroscience* 86, 903-911 (1998)

101. C. Bruder, M. Hagleitner, G. Darlington, I. Mohsenipour, R. Wurzner, I. Hollmuller, H. Stoiber, C. Lass-Florl, M.P. Dierich and C. Speth:. HIV-1 induces complement factor C3 synthesis in astrocytes and neurons by modulation of promoter activity. *Mol Immunol.* 4013), 949-946 (2004)

102. P.J.H. Jongen, W.H. Doersburg, J.L.M. Ibrahim-Stappers, W.A.J.G. Lemmens, O.R. Hommes and K.J.B. Lamers: Cerebrospinal fluid C3 and C4 indexes in immunological disorders of the central nervous system. *Acta Neurol Scand.* 101, 116-121 (2000)

103. L. Poluektova, T. Moran, M. Zelivyanskaya, S. Swindells, H.E. Gendelman and Y. Persidsky: The regulation of alpha chemokines during HIV-1 infection and leukocyte activation: relevance for HIV-1-associated dementia. *J Neuroimmunol.* 120, 112-128 (2001)

104. S.L. Wesselingh, C. Power, J.D. Glass, W.R. Tyor, J.C. McArthur, J.M. Farber, J.W. Griffin and D.E. Griffin: Intracerebral cytokine messenger RNA expression in acquired immunodeficiency syndrome dementia. *Ann Neurol.* 33, 576-82 (1993)

105. J. Wang, K. Crawford, M. Yuan, H. Wang, P.R. Gorry and D. Gabzuzda: Regulation of CC chemokine receptor 5 and CD4 expression and human immunodeficiency virus type 1 replication in human macrophages and microglia by T helper type 2 cytokines. *J Infect Dis.* 185, 885-897. (2002)

106. J. Coyle-Rink, T. Sweet, S. Abraham, B. Sawaya, O. Batuman, K. Khalili and S. Amini: Interaction between TGFbeta signaling proteins and C/EBP controls basal and Tat-mediated transcription of HIV-1 LTR in astrocytes. *Virology* 299, 240-247 (2002)

107. N. Janabi, M. Di Stefano, C. Wallon, C. Hery, F. Chiodi and M. Tardieu: Induction of human immunodeficiency virus type 1 replication in human glial cells after proinflammatory cytokines stimulation: effect of IFNgamma, IL1beta, and TNFalpha on differentiation and chemokine production in glial cells. *Glia* 23, 304-15 (1998)

108. Y. Han, J. Wang, T. He and R.M. Ransohoff,: TNF-alpha down-regulates CXCR4 expression in primary murine astrocytes. *Brain Res.* 888, 1-10 (2001)

109. D. Weissman, R.L. Rabin, J. Arthos, A. Rubbert, M. Dybul, R. Swofford, S. Venkatesan, J.M. Farber and A.S. Fauci:. Macrophage-tropic HIV and SIV envelope proteins induce a signal through the CCR5 chemokine receptor. *Nature* 389, 981-985 (1997)

110. C. Lee, Q.H. Liu, B. Tomkowicz, Y. Yi, B.D. Freedman and R.G. Collman: Macrophage activation through CCR5- and CXCR4-mediated gp120-elicited signaling pathways. *J Leukoc Biol.* 74, 676-82 (2003)

111. R. Kitai, M.L. Zhao, N. Zhang, L.L. Hua and S.C. Lee: Role of MIP-1beta and RANTES in HIV-1 infection of microglia: inhibition of infection and induction by IFNbeta. *J Neuroimmunol.* 110, 230-239 (2000)

112. D. Langford, V.J. Sanders, M. Mallory, M. Kaul and E. Masliah: Expression of stromal cell-derived factor 1alpha protein in HIV encephalitis. *J Neuroimmunol.* 127, 115-26 (2002)

113. H.G. Rus, L.M. Kim, F.I. Niculescu and M.L. Shin: Induction of C3 expression in astrocytes is regulated by cytokines and Newcastle Disease Virus. *J. Immunol.* 148, 928-933 (1992)

114. A. Thomas, P. Gasque, D. Vaudry, B. Gonzalez and M. Fontaine: Expression of a complete and functional complement system by human neuronal cells in vitro. *Int. Immunol.* 12, 1015-1023 (2000)

115. C. Speth, B. Jöbstl, M. Barcova and M.P. Dierich: HIV-1 envelope protein gp41 modulates expression of interleukin-10 and chemokine receptors on monocytes, astrocytes and neurones. *AIDS* 14, 629-623 (2000)

116. C. Brodie: Differential effects of Th1 and Th2 derived cytokines on NGF synthesis by mouse astrocytes. *FEBS Lett.* 394, 117-120 (1996)

117. C.U. Kloss, G.W. Kreutzberg and G. Raivich: Proliferation of ramified microglia on an astrocyte monolayer: characterization of stimulatory and inhibitory cytokines. *J Neurosci Res.* 492), 248-54 (1997)

118. D. Schols and E. De Clercq: Human immunodeficiency virus type 1 gp120 induces anergy in human peripheral blood lymphocytes by inducing interleukin-10 production. *J Virol.* 70, 4953-4960 (1996)

119. Z.F. Rosenberg and A.S. Fauci: Immunopathogenic mechanisms of HIV infection: cytokine induction of HIV expression. *Immunol Today* 11, 176-180 (1990)