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Chapter Twenty-Eight

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Nature killer cells in the central nervous system

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CHAPTER CONTENTS

A moment of realization is worth a thousand prayers.

From the movie *Natural Born Killers*, 1994

ABSTRACT

Natural killer (NK) cells, a prominent component of the innate immune system, are large granular lymphocytes that respond rapidly to a variety of insults via cytokine secretion and cytolytic activity. Recently, there has been growing insight into the biological functions of NK cells, in particular into their roles in infection, tumour surveillance and autoimmunity. Under these pathophysiological circumstances, NK cells readily home to the central nervous system (CNS) tissues to combat infection and presumably to curb progression of tumours. Bystander neuronal and/or glial cell damage can occur in this setting. Paradoxically, NK cells appear to have an inhibitory role for autoimmune responses within the CNS. As in the periphery, NK cells act in concert with T cells and other lymphocytes responsible for CNS pathology and immune regulation. Insights into the molecular signals and pathways governing the diverse biological effects of NK cells are keys for designing NK cell-based therapy for CNS infections, tumours and autoimmune diseases.

KEY WORDS

Tumour, Infection, Autoimmunity, Myelin, Glia, Herpes, Oligodendrocyte

Homing of NK cells into the CNS

The central nervous system (CNS) is an immune privileged organ because of the lack of endogenous dendritic cells (DCs) (Ransohoff et al., 2003). In many pathological conditions including stroke, traumatic injury, encephalitis, and demyelinating autoimmune disorders, however, a massive infiltration of peripheral immune cells occurs in the CNS. Although natural killer (NK) cell-related gene expression is readily detected within the CNS (Bryceson et al., 2005; Lund et al., 2007), evidence of direct demonstration of the presence of NK

cells in human CNS tissues is still lacking. This is partly because of the lack of suitable antibodies for staining human NK cells in situ (see Chapter 31). Visualization of NK cells in mouse brains during experimental autoimmune encephalomyelitis (EAE) was achieved using anti-NK1.1 mAb (PK136) (Hammarberg et al., 2000). Antibodies such as Ly49 have been successfully used for staining NK cells in lymphoid organs (O'Leary et al., 2006). Suitability of these antibodies for staining CNS NK cells needs to be verified in additional studies.

Although direct visualization of NK cells in CNS tissues is technically challenging, there is little doubt that NK cells, as with other types of lymphocytes, enter the CNS during inflammatory processes. In fact, it has been reported that NK cells are among the earliest recruited cells during adoptive transfer EAE (Kerfoot and Kubes, 2002; Wekerle and Fierz, 1985). Chemokine receptors such as CCR2, CCR5, CXCR3, CX3CR1 as well as lysophospholipid sphingosine 1-phosphate (S1P) are involved in the rapid NK-cell mobilization that occurs in inflammatory conditions (Ajuebor et al., 2007; Hokeness et al., 2005; Huang et al., 2006; Inngjerdingen et al., 2001; Jiang et al., 2004; Khan et al., 2006; Kveberg et al., 2002; Lavergne et al., 2003; Martin-Fontecha et al., 2004; Thapa et al., 2007; Wald et al., 2006; Walzer et al., 2007; Yu et al., 2007), and several of these chemokine receptors (CCR5, CX3CR1) are directly involved in NK cell homing to the CNS (Huang et al., 2006; Martin-Fontecha et al., 2004; Thapa et al., 2007). The biological implication of chemokine-guided homing of NK cells during CNS inflammation is discussed in greater detail in the following section.

NK cell-mediated neuron, oligodendrocyte and glial cell damage

The ability of NK cells to kill various transformed and virus-infected cells raises an important question whether direct NK cell cytolytic effects contribute to the pathogenesis of inflammatory, degenerative and autoimmune disorders of the nervous system. Neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease are characterized by the death of neurons in distinct functional neuron-anatomic systems. Multiple sclerosis (MS), on the other hand, is characterized by inflammation and demyelination within the spinal cord and brain, and axonal damage and brain atrophy also occur during the course of disease. The peripheral form of MS is Guillain–Barré syndrome, which is also characterized by demyelination and cellular infiltrates of the peripheral nervous system. Some of these diseases involve immunologic components or reactions, and some have been characterized extensively in the different systems. Autoreactive T cells and adaptive immune system components in the pathogenesis in some of these disorders are well characterized. As discussed here, evidence for direct NK cell cytolytic effects is emerging. Although the in vivo relevance of a great proportion of these studies needs to be validated, the current data emphasize the importance of NK cells either in direct cytotoxic effects or in collaboration with cells from both innate and adaptive immune systems in the initiation of these neurodegenerative and inflammatory diseases.

Neuron

The NK cell-dependent death of sympathetic neurons resident in the superior cervical ganglia of rats, observed after the exposure to the drug guanethidine (Hickey et al., 1992; Hougen et al., 1992), is the first in vivo disorder of the nervous system in which NK cells appear to be the dominant effector cells. The pathogenic mechanism observed appeared to represent a novel type of autoimmune reaction: an exogenously/chemically induced alteration in a specific subset of cells that was suggested to target them for direct NK cell-mediated killing.

Interestingly, neuronal cells from the peripheral system and the CNS appear to have different susceptibility to NK cell killing. Ljunggren and colleagues have carried out a series of well-designed studies addressing this puzzling phenomenon. Initially, it was demonstrated that NK cells could readily kill syngeneic dorsal root ganglia (DRG) neurons by a perforin-dependent mechanism (Backstrom et al., 2000, 2003). Ventral spinal cord neurons and hippocampal neurons of the CNS were resistant to lysis. The resistance to NK cell-mediated lysis of the latter neurons was not related to protection by MHC class I molecules, since similar β_2-micro-globulin$^{-/-}$ neurons were equally resistant to lysis (Backstrom et al., 2003).

NK cell function is tightly regulated by multiple signals transmitted via inhibitory and activating receptors. The prerequisite for NK cell killing is its activation via signalling from activating receptor ligand pathways. NK cell activation generally appears to be elicited by a distinct set of molecules that have weak homology with MHC class I molecules. The activating receptor NKG2D which differs dramatically from other NKG2 receptor proteins is of particular interest since it, in contrast to other NK cell-activating receptors, is constitutively expressed on NK cells. The endogenous ligand of NKG2D in the mouse was recently identified as retinoic acid early inducible gene-1 (RAE-1)-encoded proteins and minor histocompatibility antigen H60 (Cerwenka et al., 2000; Diefenbach et al., 2000; Malarkannan

et al., 1998). Differential expression of NKG2D and its ligand on neuronal cells from the peripheral system or the CNS appears a key mechanism underlying variable susceptibility to NK lyses. RAE-1, the product of which is a ligand for the NK cell-activating receptor NKG2D, was expressed at high levels in the DRG neurons. In contrast, RAE-1 was expressed only at very low levels in the resistant CNS-derived neurons. Blocking NK cells with anti-NKG2D antibodies inhibited NK cell-mediated killing of the DRG neurons.

These findings are important in revealing novel immunopathologic effects of several CNS diseases. Indeed, progressive motor and sensory neuropathy developed in a patient with chronic NK cell lymphocytosis (CNKL) (Noguchi et al., 2005). A sural nerve biopsy revealed infiltration of NK cells into the nerve fascicles, and demyelinating changes with secondary axonal degeneration. The infiltrating NK cells were adjacent to myelinated fibres, showing damage of Schwann cell membranes. Treatment with oral prednisolone resulted in rapid improvement of sensory disturbance and weakness with a significant decrease of NK cells in the blood and disappearance of the conduction block. These observations suggest that the infiltrating NK cells may directly damage myelin and Schwann cells, thus causing demyelination.

Since expression of NKG2D ligands is likely regulated by viral infection or transformation, and the inhibitory MHC class I expression is low or absent in the nervous system, it is plausible that a viral infection or transformation could well break the balance of activating/inhibiting activities on NK cells, and NK cell-mediated immune pathology would occur in such circumstances.

Oligodendrocyte

Human oligodendrocytes do not express MHC class II molecules; thus, direct MHC-restricted injury mediated by myelin-reactive $CD4^+$ T cells is less likely to occur. The migration of NK cells to the CNS during inflammatory responses and lack of inhibitory signal MHC class I expression within the CNS invites prediction that direct cytolytic effects of NK cells contribute to oligodendrocyte damage and demyelination to CNS diseases such as MS. Antel and colleagues demonstrated using in vitro assays that human oligodendrocytes, as well as other glial elements (astrocytes, microglia), were susceptible to injury mediated by peripheral blood-derived mononuclear cell preparations (MNCs), which were enriched for NK cells by depleting $CD3^+$, with or without $CD19^+$ cells through the use of either magnetic beads or cell sorting (Antel et al., 1998; Morse et al., 2001). Cytotoxic effects of the NK cell-enriched effectors were dependant on pre-exposure of these cells to IL-2. Furthermore, it was found that autologous oligodendrocytes were as susceptible to injury mediated by IL-2-activated NK cells as were heterogonous oligodendrocytes.

In searching for receptor ligand pathways that control the NK cell and oligodendrocyte interactions, it was found that human adult oligodendrocytes and foetal astrocytes expressed ligands for NKG2D in vitro, whereas neurons, microglia, and adult astrocytes did not (Saikali et al., 2007). Disruption of the NKG2D–NKG2D ligand interaction using blocking antibodies significantly inhibited killing of primary human oligodendrocytes mediated by activated human NK cells (Saikali et al., 2007). These results imply that NKG2D–NKG2D ligand interactions can potentially contribute to cytotoxic responses mediated by activated immune effector cells in the inflamed CNS, as observed in MS.

In the context of tissue injury that occurs in MS, the inflammatory milieu in MS lesions may provide conditions required for NK cell activation, raising the possibility that such effector cells would play a role in the pathogenesis. In addition to direct cytotoxicity, cytokine release by NK cells may also participate in tissue damage as well as in regulating T cell immune responses. Interferon-gamma (IFN-γ) is a pleiotropic cytokine produced by T cells and NK cells that has been implicated as a deleterious factor in MS, the immune-mediated demyelinating disorder. In vitro, purified developing and mature oligodendrocytes die in the presence of IFN-γ by apoptosis and necrosis, respectively. Transgenic expression of PLP/SOCS1 (proteolipid protein regulating suppressor of cytokine signalling 1), brings about diminished oligodendrocyte responsiveness to IFN-γ attributable to the targeted expression of SOCS1 in these cells (Balabanov et al., 2006). Consequently, oligodendrocytes in the PLP/SOCS1 transgenic mice are protected against the injurious effect of IFN-γ. Although both NK cells and T cells produce IFN-γ, NK cells are the principal sources of early IFN-γ production prior to T cell activation. This time kinetic might be particularly relevant for early oligodendrocyte damage during inflammation.

NK cells in infection of the CNS

Efficient early control of viral infections is determined by viral tissue tropism and rate of replication as well as the host's ability to mount an effective immune response. Cellular cytotoxicity, in particular, that of NK cells and cytotoxic T cells, is central to the early antiviral immune response. Table 28.1 illustrates several immune-deficient conditions in humans, which stem from mutations affecting NK cells (Biron et al., 1989; Gilmour et al., 2001; Markel et al., 2004; Moins-Teisserenc et al., 1999). A number of studies have demonstrated the recruitment and activation of NK cells following infection with a wide range of viruses. However, not all viral infections

Table 28.1 Genetic mutation or aberrant expression of cytokines affecting NK cells leads to infection and autoimmunity in humans

Mutations	Patient phenotype	Immune phenotype	References
IL-2R/IL-15Rβ	$NK^{-/-}$ SCID phenotype	Pronounced reduction in NK cells	Alsharifi et al. (2006)
Not known	Herpes virus infection	Absence of $CD16^{+}$ NK cells	Armstrong et al. (2001)
TAP-deficiency	Chronic infection and systemic autoimmunity	Defective $CD8^{+}$ T cell responses	Whitley (2002), Bellner et al. (2005)

SCID: combined severe immunodeficiency
TAP: transporter associated with antigen processing

are susceptible to NK-mediated clearance, and susceptibility depends upon the effector mechanisms induced. For example, the induction of both cytotoxicity and IFN-γ production by NK cells following murine cytomegalovirus (CMV) and influenza virus infection results in reduced virally induced disease and enhanced survival. Along the same time, deficient IFN-γ production by NK cells correlates with the absence of an effective innate response to lymphocytic choriomeningitis virus infection. Moreover, the organs targeted by viral infection can also influence the participation of NK cells. Indeed, it has been shown that the NK response to murine CMV is perforin-dependent within the spleen, whereas production of IFN-γ is required for viral clearance from the liver. These results indicate that the importance of the NK cell response to viral infection can depend upon multiple factors, including the tissue infected, as well as the effector mechanisms induced.

Although a number of studies have documented the possible role for NK cells in controlling CNS infection with CMV, influenza and other viruses, the following studies provide relatively direct evidence for the importance of NK cells during CNS viral infection:

Theiler's murine encephalomyelitis virus

Theiler's murine encephalomyelitis virus (TMEV) is a picornavirus. Infection of susceptible mice (SJL) with TMEV causes a biphasic disease characterized by grey matter inflammation followed by late chronic demyelination (Roos and Wollmann, 1984; Rosenthal et al., 1986). After inoculation, CNS TMEV titres were higher in SJL mice compared with C57BL/10 mice, correlating with a 50% lower NK cell activity in the SJL mice than in the C57BL/10 mice (Paya et al., 1989). Clinically, SJL mice are much more susceptible than C57BL/10 mice to TMEV. When resistant (C57BL/10) mice were depleted of NK cells using either mAb NK1.1 or polyclonal anti-asialo-GM1, TMEV induced the development of diffuse encephalitis and meningitis early in the post-infection period. However, the second phase of TMEV-induced CNS disease (demyelination) was observed only in resistant C57BL/10 mice treated with anti-asialo-GM1. Experiments with beige/beige mice of C57BL/10 background showed a mild degree of grey matter inflammation but no demyelination (Paya et al., 1989). NK cells are critical effectors in protecting against TMEV-induced grey matter disease, whereas a different population of either NK1.1–NK cells, or other activated lymphocytes, may be critical in resistance or susceptibility to demyelination.

In support of the involvement of NK cells during TMEV of the CNS, another study demonstrated that stressed mice developed clinical signs of encephalitis, thymic atrophy, and adrenal hypertrophy after infection with Theiler's virus (Welsh et al., 2004). This syndrome was associated with significantly reduced virus-induced NK cell cytotoxic activity in restrained mice at 1 day post-infection, which may account for the reduced viral clearance from the CNS.

Mouse hepatitis virus

Mouse hepatitis virus (MHV) is a coronavirus that causes infection of the CNS (Marten et al., 2001; Wang et al., 1990). Intracerebral infection of susceptible strains of mice with MHV results in an acute encephalomyelitis followed by a chronic immune-mediated demyelinating disease that is similar in pathology to the human demyelinating disease MS (Walsh et al., 2007; Zuo et al., 2006). Intracerebral infection of *RAG1*$^{-/-}$ mice with a recombinant CXCL10-expressing murine coronavirus (MHV) resulted in protection from disease and increased survival that correlated with a significant increase in recruitment and activation of NK cells within the CNS (Walsh et al., 2007). Accumulation of NK cells resulted in a reduction in viral titres that was dependant on IFN-γ secretion (Walsh et al., 2007). These results indicate that the CXCL10-guided NK cell homing to the CNS might play a pivotal role in defence following coronavirus infection of the CNS.

Semliki Forest virus

Semliki Forest virus (SFV) is a positive-stranded RNA virus (Atkins et al., 1999; Smithburn and Haddow, 1944). Infection of C57BL/6 mice with SFV leads to

pronounced CNS cellular infiltration and apoptosis of microglial and neuronal cells (Alsharifi et al., 2006). In this model, NK cells and, to a lesser degree, cytotoxic T cells are major contributors in combating SFV infection. Mice lacking the Tc cell compartment (β_2-microglobulin-deficient mice, and thus $CD8^+$ T cells) exhibit susceptibility similar to wild-type mice. Depletion of NK cells significantly delayed the mean time to death but did not prevent mortality in SFV-infected B6 mice suggesting that cytolytic activity of NK cells is detrimental, while IFN-γ production is beneficial for recovery from SFV infection (Alsharifi et al., 2006).

Herpes simplex virus

With greater than 1.6 million Americans infected annually (Armstrong et al., 2001), herpes simplex virus type 1 and 2 (HSV-1, HSV-2) are pathogens with a significant impact on public health. Typically, infection results in a life-long latent infection of the host (Halioua and Malkin, 1999; Whitley, 2002). The transmission of HSV-2 in the human population includes asymptomatic shedding of the virus even in the presence of $CD8^+$ cytotoxic T lymphocytes and the production of a viral glycoprotein that indirectly elicits NK cell death (Bellner et al., 2005; Posavad et al., 2000; Wald et al., 2000). In a mouse model of HSV-2 infection, it was shown that mice deficient in CCR5 ($CCR5^{-/-}$) displayed a significant reduction in cumulative survival following infection in comparison with wild-type HSV-2–infected controls. Associated with decreased resistance to viral infection, $CCR5^{-/-}$ mice yielded significantly more virus and expressed higher levels of tumour necrosis factor alpha (TNF-α), CXCL1, CCL2, CCL3 and CCL5 in the vagina, spinal cord, and/or brain stem than did wild-type mice. In addition, when comparing wild-type HSV-2–infected mice with $CCR5^{-/-}$ mice prior to or after infection, there were significantly more NK cells ($NK1.1^+$ $CD3^-$) residing in the brain stem and spleen of infected wild-type mice. Functionally, NK activity from cells isolated from the brain stem of HSV-2–infected wild-type mice was greater than that from HSV-2–infected $CCR5^{-/-}$ mice. Further, antibody-mediated depletion of NK cells resulted in an increase in HSV-2 levels in the vaginal, spinal cord and brain stem tissue of wild-type mice but not $CCR5^{-/-}$ mice (Kveberg et al., 2002). Collectively, the absence of CCR5 expression significantly impacts the ability of the host to control genital HSV-2 infection, inflammation and spread associated with a specific reduction in NK cell expansion, infiltration and activity in the nervous system.

Toxoplasma gondii

Congenital toxoplasmosis poses a public health problem, being capable of causing foetal death and ocular and neurological sequelae in congenitally infected children. Congenital infection occurs only when mothers first encounter *Toxoplasma gondii* (*T. gondii*) during pregnancy (Remington et al., 1994; Roberts and Alexander, 1992). Resistance to *T. gondii* is mainly mediated by type 1 cytokines, such as IFN-γ and interleukin 2 (IL-2), whereas type 2 cytokines, such as IL-4 and IL-10, are associated with increased susceptibility to infection (Hunter et al., 1996; Khan et al., 1994). Susceptibility of the pregnant host to toxoplasmosis may be attributable to a type 2-cytokine bias that is maintained during gestation (Shirahata et al., 1992). Cell-mediated immune responses involving CD4 and CD8T cells and NK cells play a protective role in *T. gondii* primary infection (Goldszmid et al., 2007; Scharton-Kersten et al., 1998; Scorza et al., 2003; Scott and Trinchieri, 1995; Subauste et al., 1992). To clarify the roles of NK cells and IFN-γ in protection against primary congenital toxoplasmosis, Abou-Bacar and colleagues (2004) used recombination activating gene 2 knockout (KO) ($RAG\text{-}2^{-/-}$) mice, which lack T and B lymphocytes, in comparison with the wild-type BALB/c model. $RAG\text{-}2^{-/-}$ mice had a significantly lower risk of foetal toxoplasmosis than BALB/c mice. This protection was associated with an increased number of maternal NK cells, IFN-γ secretion by spleen cells, and decreased parasitemia. In the $RAG\text{-}2^{-/-}$ mice, NK cell depletion increased the rate of foetal infection. These data suggest that a partially protective immunity against congenital toxoplasmosis is achieved owing to the increased number of NK cells in $RAG\text{-}2^{-/-}$ mice (Abou-Bacar et al., 2004). Protective effect of NK cells was confirmed in another study using the SCID model (Kang and Suzuki, 2001).

NK cells and tumour immune surveillance of the CNS

The innate immune system plays an instrumental role in generating and directing the adaptive immune responses (Shi et al., 2001). NK cells represent a critical first line of defence against malignant transformation. Earlier results by Karre and Ljunggren demonstrated that NK cells can preferentially kill and reject cells that fail to express 'self' MHC class I molecules (Karre et al., 1986). These findings led to the formation of the famous 'missing self hypothesis' (Ljunggren and Karre, 1990). Over the years, the missing self hypothesis has been repeatedly demonstrated in a variety of experimental tumour systems by different groups of investigators,

and a number of molecular pathways governing the interactions of NK cell–target cells have been revealed. Surveillance against 'missing self' may thus be one, but not the only function of NK cells (Ljunggren and Malmberg, 2007).

Non-surgical resectable tumours within the CNS constitute significant challenges for physicians. Furthermore, studies have documented frequent immune system defects in intracranial tumour-bearing patients and an inability of certain lymphocyte subset to mediate anti-tumour effector functions in the CNS.

Metastatic melanoma

Malignant melanoma is notorious for metastasis to discrete locations such as testis and brain. Malignant melanoma is the third most common type of cancer that metastasizes to the brain (Prins et al., 2006), which presents clinicians with few treatment options. Although nearly a dozen melanoma antigens specifically recognized by T cells have been identified, melanoma cells are still able to avoid immune destruction in most instances. Because the generation of an effective anti-tumour immune response requires both the presence of foreign antigen and a costimulatory molecule or signal, tumour cells displaying tumour antigens may avoid immune detection because of the absence of appropriate costimulation. Thus, anti-tumour immune responses might be achieved by more effective local delivery of costimulatory molecules. Activation and expansion of NK cells may independently lyse tumour cells, or provide T cells with costimulatory molecules including cytokines, and overall enhance antigen presentation to T cells.

Several attempts have been made in an effort to use NK cells to target CNS melanoma. The specific receptor for IL-2 on NK cells allows several approaches to deliver IL-2 intrathecally and activate NK cells. Ewend and associates carried out a study in C57BL/6 mice that were simultaneously given intracranial injections of tumour and of irradiated B16F10 melanoma cells transduced to secrete IL-2 (Prins et al., 2006). IL-2 therapy generated antitumour responses capable of extending the survival of animals that received simultaneous intracranial tumour challenges either locally or at distant sites in the brain. Non-transduced melanoma cells had little effect. Elimination of T-cell and NK subsets using gene KO mice and antibody-depletion techniques demonstrated that NK cells were most important for the initial anti-tumour response, whereas $CD4^+$ T cells were not necessary. These studies demonstrate that local IL-2 therapy in the brain not only generates an immediate local antitumour immune response, but also establishes long-term immunologic memory capable of eliminating subsequent tumour challenges within and outside of the CNS. Furthermore, the antitumour response to paracrine IL-2 in the brain differed significantly from that in the flank, suggesting that the intrinsic CNS cells involved in initiating immunity within the brain have different cytokine requirements from their peripheral counterparts.

Using the same model, a recent study showed that DCs administration induced dramatic anti-tumour immune protection in CD8α KO mice that were challenged with B16 melanoma both subcutaneously and in the brain (Ewend et al., 2000). The CNS anti-tumour immunity was dependant on both $CD4^+$ T cells and NK cells. Administration of non-Ag-loaded, immature DC resulted in significant $CD4^+$ T cell and NK cell expansion in the draining lymph nodes at 6 days post-vaccination, which persisted for 2 weeks. Finally, Ag-loaded DC administration in CD8α KO mice was associated with robust infiltration of $CD4^+$ T cells and NK cells into the brain tumour parenchyma (Ewend et al., 2000).

Glioma

Glioma cells interfere with anti-tumour immune responses by expressing immune inhibitory cell surface molecules, such as HLA-G, or by releasing soluble immunosuppressants such as transforming growth factor (TGF-β). They rarely metastasize outside the brain, raising the possibility of immune-mediated control of these cells outside, but not inside, the brain.

IL-2, as well as growth hormone, is potent in enhancing NK cell activity against glioma both in human trials and in several experimental systems (Eisele et al., 2006; Hayes et al., 1995; Shimizu et al., 2005; Wischhusen et al., 2005). As receptors governing NK cell action and effector functions are being elucidated, more sophisticated means of manipulating NK cells have been generated. As noted above, NKG2D is a powerful, activating NK cell receptor (Wischhusen et al., 2005). Accordingly, activating the innate immune system by forcing glioma cells to express danger signals such as NKG2D ligands is a promising strategy of immunotherapy for these tumours. The remaining challenges would be to down-regulate HLA-E expression on glioma cells and suppress production of TGF-β by glioma. Both HLA-E and TGF-beta can down-regulate NKG2D expression on glioma, which enable these tumour cells to escape NK cell surveillance.

Other CNS tumours

Various studies have documented the role of NK cells in surveillance and suppression of other type of CNS tumours including medulloblastoma (Castriconi et al.,

Table 28.2 NK cells in CNS pathology

CNS pathology	Functions	Mechanisms	References
Viral infection			
TMEV	Suppress viral infection	Not studied	Paya et al. (1989)
Theiler's virus	Inhibit viral replication	Kill viral infected cells	Welsh et al. (2004)
Mouse hepatitis virus	Inhibit viral replication	Not studied	Walsh et al. (2007), Zuo et al. (2006)
Herpes simplex virus	Confine viral infection	IFN-γ production by NK cells	Alsharifi et al. (2006)
Semliki Forest virus	Inhibit viral replication	Cytotoxicity IFN-γ production	Kveberg et al. (2002)
Tumours			
CNS melanoma	Suppress tumour growth	NK cytotoxicity	Prins et al. (2006)
Glioma	Suppress tumour growth	TGF-β production and NKG2D activation	Wischhusen et al. (2005)
Astrocytoma	NO significant role	–	Kang et al. (2004)
Medullbostoma	NO significant role	–	Castriconi et al. (2007)
Inflammation			
Multiple sclerosis and EAE	Suppression	Kill CNS APC inhibit myelin-reactive T cells	Zhang et al. (1997), Huang et al. (2006), Bielekova et al. (2006)

TMEV: Theiler's Murine Encephalomyelitis Virus
EAE: Experimental Autoimmune Encephalomyelitis

2007), astrocytoma and neuroblastoma (Kang et al., 2004). On the other hand NK cells appear to have little, if any, role in suppressing CNS lymphomas (Yamasaki et al., 2003).

Clearly, cumulative evidence suggests that NK cells play a role in curbing malignant transformation and progression of many primary and metastatic CNS tumours (Table 28.2). Direct cytotoxic effects and collaboration with T cells and other immune cells are required to achieve these functions (Table 28.2). Effective therapies harnessing NK cells will be facilitated through understanding of the molecular signalling pathways that will be governing NK cell activation, expansion and maintenance. Specific anatomical factions within the CNS should also be considered. Furthermore, effort must be taken in suppressing the capacity of certain tumours to down-regulate activating signals and production of inhibitory proteins against NK cells.

Regulatory functions of NK cells in CNS inflammation and autoimmunity

During CNS infection, cytolytic activity of NK cells contributes to elimination of viral and bacterial infected cells and controls the magnitude of inflammation. Debris from neuronal and/or glial cell death is taken up by antigen-presenting cells (APCs) and presentation to T cells. Cytokine (IFN-γ) secretion by NK cells increases MHC class II expression by APC and, thus, favours generation of Th1 type of T helper cells. Thus, NK cells function not only as the initial line of host defence, but also as fuel to the generation of adaptive immune responses. Overall, NK cells are expected to boost immune response within the CNS. Paradoxically, emerging evidence suggests that NK cells can inhibit CNS inflammation and control the magnitude of autoimmunity (Table 28.2).

MS is a classic autoimmune disease characterized by extensive CNS inflammation and immune-mediated destruction of myelin. Consequently, the function of myelin sheaths becomes compromised and neurological impairment occurs. The pathogenesis of MS is mirrored, in part, in EAE, which can be induced in susceptible strains of mice with neuron-antigens and complete Freund's adjuvant. The roles of NK cells in the pathogenesis of MS and EAE have been investigated. In patients with MS, NK cells (CD56 and CD57) are present in the peripheral blood with reduced numbers and cytolytic activity (Shibatomi et al., 2001; Trinchieri, 1989). This finding is not unique to MS and similar phenotype of NK cells have been documented in many other types of autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, myasthenia gravis, etc (Shibatomi et al., 2001). In parallel, patients with MS and other autoimmune diseases have defective functions of 'regulatory cells', including NKT cells and $CD4^+CD25^+$ regulatory T cells (Treg) (La Cava et al., 2006). A reduced number and/or compromised function of NK cells, NKT cells and Treg cells invite a hypothesis that autoimmunity is associated with a result of global defective regulatory cell functions in these patients.

This hypothesis has been tested in several EAE models. Because gene encoding NK cells cannot be targeted by the current technology, several depleting antibodies have been used to study the function of NK cells in vivo. Several groups have utilized anti-NK1.1 mAb and observed that depletion of NK cells by injecting anti-NK1.1 mAb leads to exacerbation of EAE (Zhang et al., 1997). Apparently, both peripheral and CNS NK cells are absent in this experimental system. It is, therefore, not possible to differentiate the role of NK cells in the periphery and in the CNS.

NK cell homing to CNS is controlled by a specific chemokine receptor ligand pathway involving CX3CR1 and fractalkine (CX3CL1). CX3CR1 is expressed almost exclusively by CNS glial cells (Boehme et al., 2000; Cardona et al., 2006). Thus, germ-line deletion of CX3CR1 leads to impaired homing of NK cells to the CNS. This model would be ideal in addressing CNS inflammation/autoimmunity in relation to NK cells. Interestingly, upon immunization, CX3CR1-deficient mice with reduced NK cells in the CNS and intact NK cells in the periphery developed their wild-type controls. Thus, lack of CNS NK cells alone is sufficient to cause exacerbated CNS inflammation and autoimmunity (Huang et al., 2006). It is also conceivable that chemokine guided NK cell homing to CNS might serve as pathway that can be therapeutically targeted (Figure 28.1).

Infection is suggested to play a role triggering the initiation of MS in some patients (Bendelac and Medzhitov, 2002; Pulendran and Ahmed, 2006; Shirahata et al., 1992). The use of complete Freund's adjuvant in the induction of EAE may mimic this process (Fearon and Locksley, 1996). In these patients or in EAE animals, NK cells may contribute to the demyelination through bystander damage while controlling the infection. Once infection is controlled, NK cells may function to inhibit the excessive (auto) immune responses elicited by pathogens. The immune system may use NK cell as a versatile regulator to tune its capacity in combating infection and avoiding autoimmunity.

The mechanism underlying this unique role for NK cells within the CNS during EAE is still elusive. A close survey of the literature reveals multiple steps where NK cells can regulate inflammation and intervene in the loss of self-tolerance. Importantly, the findings also caution against inferring a similar role for NK cells in all types of autoimmune phenomena or during separate stages of the same disease (Flodstrom et al., 2002; Yokoyama and Plougastel, 2003). NK cells can both promote and inhibit autoreactive T cells. These possibilities have been extensively reviewed recently (Shi and Van Kaer, 2006). The specific CNS anatomical location, as reflected by diverse CNS APCs and multiple antigens may also influence the outcome of autoimmunity. As with EAE, it appears that NK cells control T cell proliferation in an antigen non-specific manner, both in the periphery and within the CNS (Shi, et al., unpublished). Recently, it has been demonstrated that human NK cells kill resting but not activated microglia via NKG2D- and NKp46-mediated recognition (Lünemann et al., 2008). This study emphasizes the potential importance of interactions between NK cells and CNS resident APCs. However, whether the regulatory effects of NK cells can be attributed to the action of NK cells on APCs, directly on T cells, or both, is not known and currently under investigation.

Summary and future research directions

NK cells readily accumulate in homing to CNS tissues under the pathophysiological situations. This process is tightly controlled by a number of chemokines and chemokine receptors. There is ample of evidence that

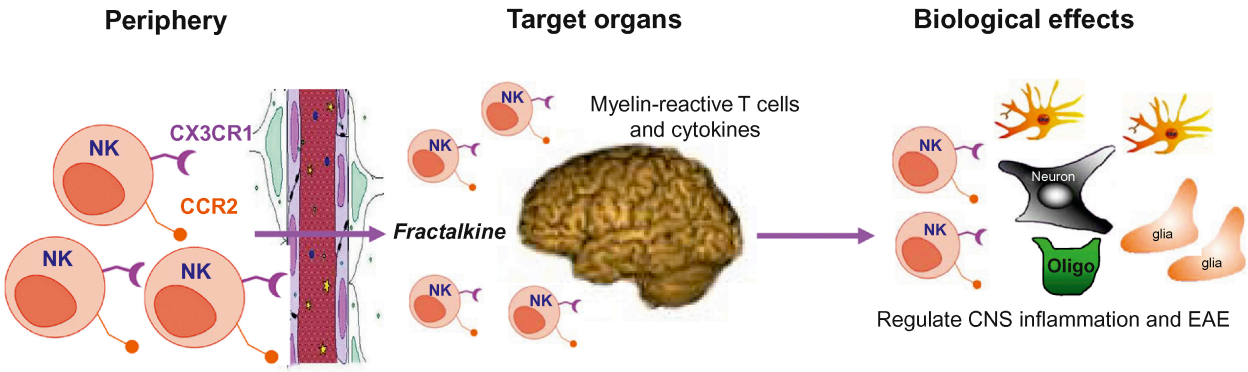

Figure 28.1 • Chemokine-guided recruitment of NK cells as in the CNS. Inflammatory responses as seen in EAE or MS may result in the production of chemokines, in particular fractalkine by microglia. Fractalkine recruits NK cells to the CNS. Subsequently, NK cells may control the magnitude of CNS inflammation and severity of EAE via several pathways: (1) directly kill APCs and interfere with reactivation of myelin-reactive T cells; (2) directly kill myelin-reactive T cells; altered expression of MHC class I on those APCs and T cells may trigger the killing; (3) suppress differentiation of T helper cells. This can be achieved by depleting cells which produce Th cell-polarizating cytokines, or cytokines produced by NK cells themselves.

NK cells within the CNS contribute to the control of infections and might limit progression of certain tumours. Bystander neuronal and/or glial cell damage can occur. In certain autoimmune conditions of the CNS, NK cells appear to have an inhibitory role. Activation and expansion of NK cells through engaging IL-2 receptors on NK cells not only inhibit several CNS tumours, but also might slow the progression of MS and other autoimmune diseases (Bielekova et al., 2006; Li et al., 2005). Furthermore, the ability of IFN-α and IFN-β to ameliorate MS in humans and IFN-γ to inhibit EAE in mice may reflect the ability of these cytokines to transiently activate NK-dependent regulatory responses. However, because IFN treatment also upregulates Qa-1 expression on T cells (Ota et al., 2005), the short duration and usually modest nature of these therapeutic effects may reflect a Qa-1-dependent decrease in NK cell activation and associated immunoregulatory activity (Lu et al., 2007).

Disassociation of disease-inhibiting versus disease-promoting effects of NK cells is a key to harnessing NK cells for therapeutic purposes. To achieve this goal, a generation of genetic models with selective NK cell deficiency, and development of reagents (antibodies) for visualizing subsets of NK cells in situ will be necessary. Optimization of methods to produce NK cells in large quantities for therapeutic usage is also important. Clearly, understanding the molecular signals and pathways governing these differential biological effects of NK cells as well as their cross talk with T cells is key to designing NK cell-based therapy for CNS infections, tumours and autoimmune diseases.

Acknowledgements

We thank Dr R. Liu, Dr W. Piao, and Dr. Dayao and Ms. Linda Swanson for their wonderful assistance in preparation of this chapter. The Muscular Dystrophy Association, the Barrow Neurological Foundation, and the National Institutes of Health support the authors' work in the laboratories.

References

Abou-Bacar, A., Thompson, R.C. and Anderson, R. (2004). Role of NK cells and gamma interferon in transplacental passage of *Toxoplasma gondii* in a mouse model of primary infection. Infect Immun **72**, 1397–1401.

Ajuebor, M.N., Wondimu, Z. and Hogaboam, C.M. (2007). CCR5 deficiency drives enhanced natural killer cell trafficking to and activation within the liver in murine T cell-mediated hepatitis. Am J Pathol **170**, 1975–1988.

Alsharifi, M., Lobigs, M. and Simon, M.M. (2006). NK cell-mediated immunopathology during an acute viral infection of the CNS. Eur J Immunol **36**, 887–896.

Antel, J.P., McCrea, E. and Ladiwala, U. (1998). Non-MHC-restricted cell-mediated lysis of human oligodendrocytes in vitro: relation with CD56 expression. J Immunol **160**, 1606–1611.

Armstrong, G.L., Schillinger, J. and Markowitz, L. (2001). Incidence of herpes simplex virus type 2 infection in the United States. Am J Epidemiol **153**, 912–920.

Atkins, G.J., Sheahan, B.J. and Liljestrom, P. (1999). The molecular pathogenesis or Semliki Forest virus: a model virus made useful? J Gen Virol **80**(Pt 9), 2287–2297.

Backstrom, E., Chambers, B.J. and Ho, E.L. (2003). Natural killer cell-mediated lysis of dorsal root ganglia neurons via RAE1/NKG2D interactions. Eur J Immunol **33**, 92–100.

Backstrom, E., Chambers, B.J. and Kristensson, K. (2000). Direct NK cell-mediated lysis of syngenic dorsal root ganglia neurons in vitro. J Immunol **165**, 4895–4900.

Balabanov, R., Strand, K. and Kemper, A. (2006). Suppressor of cytokine signaling 1 expression protects oligodendrocytes from the deleterious effects of interferon-gamma. J Neurosci **26**, 5143–5152.

Bellner, L., Thoren, F. and Nygren, E. (2005). A proinflammatory peptide from herpes simplex virus type 2 glycoprotein G affects neutrophil, monocyte, and NK cell functions. J Immunol **174**, 2235–2241.

Bendelac, A. and Medzhitov, R. (2002). Adjuvants of immunity: harnessing innate immunity to promote adaptive immunity. J Exp Med **195**, F19–F23.

Bielekova, B., Catalfamo, M. and Reichert-Scrivner, S. (2006). Regulatory CD56(bright) natural killer cells mediate immunomodulatory effects of IL-2Ralpha-targeted therapy (daclizumab) in multiple sclerosis. Proc Natl Acad Sci U S A **103**, 5941–5946.

Biron, C.A., Byron, K.S. and Sullivan, J.L. (1989). Severe herpesvirus infections in an adolescent without natural killer cells. N Engl J Med **320**, 1731–1735.

Boehme, S.A., Lio, F.M. and Maciejewski-Lenoir, D. (2000). The chemokine fractalkine inhibits Fas-mediated cell death of brain microglia. J Immunol **165**, 397–403.

Bryceson, Y.T., Foster, J.A. and Kuppusamy, S.P. (2005). Expression of a killer cell receptor-like gene in plastic regions of the central nervous system. J Neuroimmunol **161**, 177–182.

Cardona, A.E., Pioro, E.P. and Sasse, M.E. (2006). Control of microglial neurotoxicity by the fractalkine receptor. Nat Neurosci **9**, 917–924.

Castriconi, R., Dondero, A. and Negri, F. (2007). Both CD133+ and CD133– medulloblastoma cell lines express ligands for triggering NK receptors and are susceptible to NK-mediated cytotoxicity. Eur J Immunol **37**, 3190–3196.

Cerwenka, A., Bakker, A.B. and McClanahan, T. (2000). Retinoic acid early inducible genes define a ligand family for the activating NKG2D receptor in mice. Immunity **12**, 721–727.

Diefenbach, A., Jamieson, A.M. and Liu, S.D. (2000). Ligands for the murine NKG2D receptor: expression by tumor cells and activation of NK cells and macrophages. Nat Immunol **1**, 119–126.

Eisele, G., Wischhusen, J. and Mittelbronn, M. (2006). TGF-beta and metalloproteinases differentially suppress NKG2D ligand surface

expression on malignant glioma cells. Brain **129**, 2416–2425.

Ewend, M.G., Thompson, R.C. and Anderson, R. (2000). Intracranial paracrine interleukin-2 therapy stimulates prolonged antitumor immunity that extends outside the central nervous system. J Immunother **23**, 438–448.

Fearon, D.T. and Locksley, R.M. (1996). The instructive role of innate immunity in the acquired immune response. Science **272**, 50–53.

Flodstrom, M., Shi, F.D. and Sarvetnick, N. (2002). The natural killer cell—friend or foe in autoimmune disease? Scand J Immunol **55**, 432–441.

Gilmour, K.C., Fujii, H. and Cranston, T. (2001). Defective expression of the interleukin-2/interleukin-15 receptor beta subunit leads to a natural killer cell-deficient form of severe combined immunodeficiency. Blood **98**, 877–879.

Goldszmid, R.S., Bafica, A. and Jankovic, D. (2007). TAP-1 indirectly regulates CD4+T cell priming in *Toxoplasma gondii* infection by controlling NK cell IFN-gamma production. J Exp Med **204**, 2591–2602.

Halioua, B. and Malkin, J.E. (1999). Epidemiology of genital herpes—recent advances. Eur J Dermatol **9**, 177–184.

Hammarberg, H., Lidman, O. and Lundberg, C. (2000). Neuroprotection by encephalomyelitis: rescue of mechanically injured neurons and neurotrophin production by CNS-infiltrating T and natural killer cells. J Neurosci **20**, 5283–5291.

Hayes, R.L., Koslow, M. and Hiesiger, E. M. (1995). Improved long term survival after intracavitary interleukin-2 and lymphokine-activated killer cells for adults with recurrent malignant glioma. Cancer **76**, 840–852.

Hickey, W.F., Ueno, K. and Hiserodt, J.C. (1992). Exogenously-induced, natural killer cell-mediated neuronal killing: a novel pathogenetic mechanism. J Exp Med **176**, 811–817.

Hokeness, K.L., Kuziel, W.A., Biron, C. A. and Salazar-Mather, T.P. (2005). Monocyte chemoattractant protein-1 and CCR2 interactions are required for IFN-alpha/beta-induced inflammatory responses and antiviral defense in liver. J Immunol **174**, 1549–1556.

Hougen, H.P., Thygesen, P. and Christensen, H.B. (1992). Effect of immunosuppressive agents on the guanethidine-induced sympathectomy in athymic and euthymic rats. Int J Immunopharmacol **14**, 1113–1123.

Huang, D., Shi, F.D. and Jung, S. (2006). The neuronal chemokine CX3CL1/fractalkine selectively recruits NK cells that modify experimental autoimmune encephalomyelitis within the central nervous system. FASEB J **20**, 896–905.

Hunter, C.A., Suzuki, Y. and Subauste, C.S. (1996). Cells and cytokines in resistance to *Toxoplasma gondii*. Curr Top Microbiol Immunol **219**, 113–125.

Inngjerdingen, M., Damaj, B. and Maghazachi, A.A. (2001). Expression and regulation of chemokine receptors in human natural killer cells. Blood **97**, 367–375.

Jiang, D., Liang, J. and Hodge, J. (2004). Regulation of pulmonary fibrosis by chemokine receptor CXCR3. J Clin Invest **114**, 291–299.

Kang, H. and Suzuki, Y. (2001). Requirement of non-T cells that produce gamma interferon for prevention of reactivation of *Toxoplasma gondii* infection in the brain. Infect Immun **69**, 2920–2927.

Kang, S.G., Ryu, C.H. and Jeun, S. (2004). Lymphokine activated killer cells from umbilical cord blood show higher antitumor effect against anaplastic astrocytoma cell line (U87) and medulloblastoma cell line (TE671) than lymphokine activated killer cells from peripheral blood. Childs Nerv Syst **20**, 154–162.

Karre, K., Ljunggren, H.G. and Piontek, G. (1986). Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defence strategy. Nature **319**, 675–678.

Kerfoot, S.M. and Kubes, P. (2002). Overlapping roles of P-selectin and alpha 4 integrin to recruit leukocytes to the central nervous system in experimental autoimmune encephalomyelitis. J Immunol **169**, 1000–1006.

Khan, I.A., Matsuura, T. and Kasper, L.H. (1994). Interleukin-12 enhances murine survival against acute toxoplasmosis. Infect Immun **62**, 1639–1642.

Khan, I.A., Thomas, S.Y. and Moretto, M. M. (2006). CCR5 is essential for NK cell trafficking and host survival following *Toxoplasma gondii* infection. PLoS Pathog **2**, e49.

Kveberg, L., Bryceson, Y. and Inngjerdingen, M. (2002). Sphingosine 1 phosphate induces the chemotaxis of human natural killer cells. Role for heterotrimeric G proteins and phosphoinositide 3 kinases. Eur J Immunol **32**, 1856–1864.

La Cava, A., Van Kaer, L. and Fu Dong, S. (2006). CD4+ CD25+ Tregs and NKT cells: regulators regulating regulators. Trends Immunol **27**, 322–327.

Lavergne, E., Combadiere, B. and Bonduelle, O. (2003). Fractalkine mediates natural killer-dependent antitumor responses in vivo. Cancer Res **63**, 7468–7474.

Li, Z., Lim, W.K. and Mahesh, S.P. (2005). Cutting edge: in vivo blockade of human IL-2 receptor induces expansion of CD56(bright) regulatory NK cells in patients with active uveitis. J Immunol **174**, 5187–5191.

Ljunggren, H.G. and Karre, K. (1990). In search of the 'missing self': MHC molecules and NK cell recognition. Immunol Today **11**, 237–244.

Ljunggren, H.G. and Malmberg, K.J. (2007). Prospects for the use of NK cells in immunotherapy of human cancer. Nat Rev Immunol **7**, 329–339.

Lu, L., Ikizawa, K. and Hu, D. (2007). Regulation of activated CD4+ T cells by NK cells via the Qa-1-NKG2A inhibitory pathway. Immunity **26**, 593–604.

Lund, T.C., Anderson, L.B. and McCullar, V. (2007). iTRAQ is a useful method to screen for membrane-bound proteins differentially expressed in human natural killer cell types. J Proteome Res **6**, 644–653.

Lünemann, A., Lunemann, J.D. and Roberts, S. (2008). Human NK cells kill resting but not activated microglia via NKG2D- and NKp46-mediated recognition. J Immunol **181**, 6170–6177.

Malarkannan, S., Shih, P.P. and Eden, P.A. (1998). The molecular and functional characterization of a dominant minor H antigen, H60. J Immunol **161**, 3501–3509.

Markel, G., Mussaffi, H. and Ling, K.L. (2004). The mechanisms controlling NK cell autoreactivity in TAP2-deficient patients. Blood **103**, 1770–1778.

Marten, N.W., Stohlman, S.A. and Bergmann, C.C. (2001). MHV infection of the CNS: mechanisms of immune-mediated control. Viral Immunol **14**, 1–18.

Martin-Fontecha, A., Thomsen, L.L. and Brett, S. (2004). Induced recruitment of NK cells to lymph nodes provides IFN-gamma for T(H)1 priming. Nat Immunol **5**, 1260–1265.

Moins-Teisserenc, H.T., Gadola, S.D. and Cella, M. (1999). Association of a syndrome resembling Wegener's granulomatosis with low surface expression of HLA class-I molecules. Lancet **354**, 1598–1603.

Morse, R.H., Seguin, R. and McCrea, E. L. (2001). NK cell-mediated lysis of autologous human oligodendrocytes. J Neuroimmunol **116**, 107–115.

Noguchi, M., Yoshita, M. and Sakai, K. (2005). Peripheral neuropathy associated with chronic natural killer cell lymphocytosis. J Neurol Sci **232**, 119–122.

O'Leary, J.G., Goodarzi, M. and Drayton, D.L. (2006). T cell- and B cell-independent adaptive immunity mediated by natural killer cells. Nat Immunol **7**, 507–516.

Ota, T., Takeda, K. and Akiba, H. (2005). IFN-gamma-mediated negative feedback regulation of NKT-cell function by CD94/NKG2. Blood **106**, 184–192.

Paya, C.V., Patick, A.K. and Leibson, P. J. (1989). Role of natural killer cells as immune effectors in encephalitis and demyelination induced by Theiler's virus. J Immunol **143**, 95–102.

Posavad, C.M., Huang, M.L. and Barcy, S. (2000). Long term persistence of herpes simplex virus-specific CD8+ CTL in persons with frequently recurring genital herpes. J Immunol **165**, 1146–1152.

Prins, R.M., Vo, D.D. and Khan Farooqi, H. (2006). NK and CD4 cells collaborate to protect against melanoma tumor formation in the brain. J Immunol **177**, 8448–8455.

Pulendran, B. and Ahmed, R. (2006). Translating innate immunity into immunological memory: implications for vaccine development. Cell **124**, 849–863.

Ransohoff, R.M., Kivisakk, P. and Kidd, G. (2003). Three or more routes for leukocyte migration into the central nervous system. Nat Rev Immunol **3**, 569–581.

Remington, J.S., McLeod, R. and Desmonts, G. (1994). Toxoplasmosis. In: Remington, J.S. and Klein, O.J. (eds) Infectious Diseases of the Fetus and New-Born Infant. Philadelphia: W.B. Saunders Co., pp. 141–267.

Roberts, C.W. and Alexander, J. (1992). Studies on a murine model of congenital toxoplasmosis: vertical disease transmission only occurs in BALB/c mice infected for the first time during pregnancy. Parasitology **104**(Pt 1), 19–23.

Roos, R.P. and Wollmann, R. (1984). DA strain of Theiler's murine encephalomyelitis virus induces demyelination in nude mice. Ann Neurol **15**, 494–499.

Rosenthal, A., Fujinami, R.S. and Lampert, P.W. (1986). Mechanism of Theiler's virus-induced demyelination in nude mice. Lab Invest **54**, 515–522.

Saikali, P., Antel, J.P. and Newcombe, J. (2007). NKG2D-mediated cytotoxicity toward oligodendrocytes suggests a mechanism for tissue injury in multiple sclerosis. J Neurosci **27**, 1220–1228.

Scharton-Kersten, T., Nakajima, H. and Yap, G. (1998). Infection of mice lacking the common cytokine receptor gamma-chain (gamma(c)) reveals an unexpected role for CD4+ T lymphocytes in early IFN-gamma-dependent resistance to *Toxoplasma gondii*. J Immunol **160**, 2565–2569.

Scorza, T., D'Souza, S. and Laloup, M. (2003). A GRA1 DNA vaccine primes cytolytic CD8(+) T cells to control acute *Toxoplasma gondii* infection. Infect Immun **71**, 309–316.

Scott, P. and Trinchieri, G. (1995). The role of natural killer cells in host–parasite interactions. Curr Opin Immunol **7**, 34–40.

Shi, F.D., Ljunggren, H.G. and Sarvetnick, N. (2001). Innate immunity and autoimmunity: from self-protection to self-destruction. Trends Immunol **22**, 97–101.

Shi, F.D. and Van Kaer, L. (2006). Reciprocal regulation between natural killer cells and autoreactive T cells. Nat Rev Immunol **6**, 751–760.

Shibatomi, K., Ida, H. and Yamasaki, S. (2001). A novel role for interleukin-18 in human natural killer cell death: high serum levels and low natural killer cell numbers in patients with systemic autoimmune diseases. Arthritis Rheum **44**, 884–892.

Shimizu, K., Adachi, K. and Teramoto, A. (2005). Growth hormone enhances natural killer cell activity against glioma. J Nippon Med Sch **72**, 335–340.

Shirahata, T., Muroya, N. and Ohta, C. (1992). Correlation between increased susceptibility to primary *Toxoplasma gondii* infection and depressed production of gamma interferon in pregnant mice. Microbiol Immunol **36**, 81–91.

Smithburn, K.C. and Haddow, A.J. (1944). Semliki Forest virus I isolation and pathogenic properties. J Immunol **49**, 141–157.

Subauste, C.S., Dawson, L. and Remington, J.S. (1992). Human lymphokine-activated killer cells are cytotoxic against cells infected with *Toxoplasma gondii*. J Exp Med **176**, 1511–1519.

Thapa, M., Kuziel, W.A. and Carr, D.J. (2007). Susceptibility of CCR5-deficient mice to genital herpes simplex virus type 2 is linked to NK cell mobilization. J Virol **81**, 3704–3713.

Trinchieri, G. (1989). Biology of natural killer cells. Adv Immunol **47**, 187–376.

Wald, A., Zeh, J. and Selke, S. (2000). Reactivation of genital herpes simplex virus type 2 infection in asymptomatic seropositive persons. N Engl J Med **342**, 844–850.

Wald, O., Weiss, I.D. and Wald, H. (2006). IFN-gamma acts on T cells to induce NK cell mobilization and accumulation in target organs. J Immunol **176**, 4716–4729.

Walsh, K.B., Edwards, R.A. and Romero, K.M. (2007). Expression of CXC chemokine ligand 10 from the mouse hepatitis virus genome results in protection from viral-induced neurological and liver disease. J Immunol **179**, 1155–1165.

Walzer, T., Chiossone, L. and Chaix, J. (2007). Natural killer cell trafficking in vivo requires a dedicated sphingosine 1-phosphate receptor. Nat Immunol **8**, 1337–1344.

Wang, F.I., Stohlman, S.A. and Fleming, J.O. (1990). Demyelination induced by murine hepatitis virus JHM strain (MHV-4) is immunologically mediated. J Neuroimmunol **30**, 31–41.

Wekerle, H. and Fierz, W. (1985). T cell approach to demyelinating diseases. Springer Semin Immunopathol **8**, 97–110.

Welsh, C.J., Bustamante, L. and Nayak, M. (2004). The effects of restraint stress on the neuropathogenesis of Theiler's virus infection II: NK cell function and cytokine levels in acute disease. Brain Behav Immun **18**, 166–174.

Whitley, R.J. (2002). Herpes simplex virus infection. Semin Pediatr Infect Dis **13**, 6–11.

Wischhusen, J., Friese, M.A. and Mittelbronn, M. (2005). HLA-E protects glioma cells from NKG2D-mediated immune responses in vitro: implications for immune escape in vivo. J Neuropathol Exp Neurol **64**, 523–528.

Yamasaki, T., Moritake, K. and Klein, G. (2003). Experimental appraisal of the lack of antitumor natural killer cell-mediated immunosurveillance in response to lymphomas growing in the mouse brain. J Neurosurg **98**, 599–606.

Yokoyama, W.M. and Plougastel, B.F. (2003). Immune functions encoded by the natural killer gene complex. Nat Rev Immunol **3**, 304–316.

Yu, Y.R., Fong, A.M. and Combadiere, C. (2007). Defective antitumor responses in CX3CR1-deficient mice. Int J Cancer **121**, 316–322.

Zhang, B., Yamamura, T., Takayuki, K., et al. (1997). Regulation of Experimental Autoimmune Encephalomyelitis by Natural Killer (NK) cells. J Exp Med **186**, 1677–1687.

Zuo, J., Stohlman, S.A. and Hoskin, J.B. (2006). Mouse hepatitis virus pathogenesis in the central nervous system is independent of IL-15 and natural killer cells. Virology **350**, 206–215.