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*J Immunol* 2013; 190:5355-5362; doi: 10.4049/jimmunol.1203401
http://www.jimmunol.org/content/190/11/5355

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NK Cells in Central Nervous System Disorders

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NK cells are the “founding” members of the innate lymphoid cell family. NK cells have been initially considered as nonspecific killers, whereas “primed” NK cells also abundantly produce cytokines and chemokines and likewise modulate the adaptive immune responses (7). Although NK cells can assist in dendritic cell (DC) maturation and T cell polarization, they can also prevent and limit immune responses via killing of APCs and lymphoid cells (7). These pleiotropic functions of NK cells might be related to their heterogeneity in phenotypes and functions. In mice, NK cells were shown to differentiate from CD11blow to CD11bhigh and could be further distinguished by their expression of CD27. The immature CD11b<sub>low</sub>CD27<sup>+</sup> subset has the highest proliferative capacity and is mainly found in bone marrow and lymph nodes (LN), whereas CD11b<sub>high</sub>CD27<sup>−</sup> and CD11b<sub>high</sub>CD27<sup>+</sup> NK cells present lower rates of proliferation and are found in spleen and peripheral blood (8). In humans, NK cells can be subdivided into different populations based on the relative expression of the surface markers CD16 and CD56. The mature subtype CD56<sup>dim</sup>CD16<sup>+</sup>, found mainly in peripheral blood, has reduced proliferative capacity and produces negligible amounts of cytokines but is highly cytotoxic (9). In contrast, the more immature subset, CD56<sup>high</sup>CD16<sup>−/dim</sup>, mainly present in peripheral lymphoid organs, is able to proliferate and to secrete a large range of cytokines, though it presents minimal cytotoxic capacity (10).

Although NK cell biology remains understudied in neurologic diseases, there are arguments to think that specific brain factors could modulate NK cell function and vice versa. Indeed, NK cell physiology is modulated following the circadian rhythm through the influence of neurotransmitters, such as norepinephrine, which inhibits cytotoxic function after engagement of β-adrenergic receptors (11). Alternatively, NK cells could impact CNS physiology by killing glial cells (12) or by secreting IFN-γ (13). Most knowledge about NK cells in the CNS is coming from studies of experimental autoimmune encephalomyelitis (EAE) where conflicting results are emerging, as NK cells have been shown to be either impli-

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Abbreviations used in this article: AD, Alzheimer disease; BBB, blood–brain barrier; BCSFB, blood–cerebrospinal fluid barrier; CSF, cerebrospinal fluid; DC, dendritic cell; EAE, experimental autoimmune encephalomyelitis; GBM, glioblastoma multiforme; LN, lymph node; MS, multiple sclerosis; NKCA, NK cell cytotoxic activity; NPB, nonpathological human brain; PD, Parkinson disease; TBI, traumatic brain injury.

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Viral infections. Patients with glioma are known to have impaired immune function, parallel to an increase of anti-inflammatory molecules in plasma (20). Fadul et al. (21) observed decreased absolute numbers of NK cells in PBMCs isolated from glioblastoma multiforme (GBM) patients who were treated with combined radiation therapy and temozolomide. Furthermore, NK cell frequency and their cytotoxic activity (NKCA) were shown to be affected in the blood of brain tumor patients as well as in animal glioma models (22, 23). Several factors could mediate this effect on NK cell functions. The plasma levels of anti-inflammatory molecules, such as TGF-β, were shown to be elevated in the context of brain tumors and could inhibit NK cell functions, as well as the level of expression of the activating receptor NKG2D (24). Thus, decreased expression of NKG2D was shown on NK cells that were isolated from GBM patients (24). This downmodulation of NK cell functions in brain tumors reflects a common mechanism of immune escape found in other solid tumors (25).

Infections of the CNS. Viral infections. Shortly after they were discovered, NK cells were shown to play an important role in antiviral immune responses. The rare patients completely devoid of NK cells were extremely susceptible to infections by viruses of the herpes family (26, 27). Five consecutive children with herpetic encephalitis were shown to have functional NK cell deficiencies (27). By using a mouse model of HSV1 encephalitis, Adler et al. (28) directly demonstrated that NK cells have an important role in the defense against this severe form of infection. Nonetheless, TAP-deficient patients known to have hyporesponsive NK cells rarely present with viral encephalitis (29). In contrast, TLR3–deficient patients are susceptible to HSV encephalitis, and they present impaired NK cell IFN-γ secretion following stimulation with polyinosinic-polycytidylic acid, but normal production against K562 (30), suggesting that TLR3 dysfunction could be at the origin of this disease phenotype. Additionally, it has been described that NK cells are important actors of the antiviral immune response in mouse cerebral infections by Thérier’s murine encephalomyelitis virus (31), in mouse hepatitis virus (32), and in Semliki Forest virus (4), with a rather detrimental cytotoxic function in the latter case. Regarding SIV infection in macaques (33), it has been shown that the intensity of NK cell cytotoxicity was inversely related to the severity of CNS lesions.

Bacterial infections of the CNS. Only a few studies addressed the role of NK cells in bacterial infections of the CNS. Hayashi et al. (34) developed a nonlethal mouse model of CNS listeriosis. Under these conditions, NK cells were identified as the main source of bacterial growth-limiting IFN-γ in the early phase of infection. Mice are relatively protected from Listeria neuroinvasion as long as they have NK cells and can respond to IFN-γ (35). In contrast, NK cell–derived IFN-γ was found to be responsible for the immune pathology associated with Streptococcus pneumoniae meningitis, whereas IFN-γ KO mice are resistant to severe forms of this disease (36).

Cerebral malaria. In 1986, Stach et al. (37) described that NK cell cytotoxic function was normal in benign uncomplicated malaria, whereas it was severely decreased in children with cerebral malaria. In a mouse model of malaria induced by Plasmodium berghei ANKA, it was shown that the NK cell complex genetically influences pathogenesis, that NK cells are necessary for both disease pathogenesis and control of parasitemia, and that NK cells infiltrate the brain of mice with cerebral malaria (38). The same group demonstrated that a reciprocal activation of NK cells and DCs is implicated in the development of inflammatory pathways during malaria (39). With IFN-γ being known as a crucial factor for the development of cerebral pathology in malaria, Villegas-Mendez et al. (40) identified NK cells as the early source of this cytokine, before T cells take center stage. Overall, in CNS infections, NK cells can be beneficial or detrimental according to the experimental model used. An emerging concept states that neuroinflammation following infection may play an important role in the etiology of neurodegenerative diseases (41).

Neurodegenerative diseases. Alzheimer disease and Parkinson disease. Alzheimer (AD) and Parkinson (PD) diseases are age-related neurodegenerative conditions characterized by neuronal loss in hippocampus and substantia nigra, respectively. There are concepts supporting that AD and PD pathogenesis could be originally related to systemic immune system dysfunction (42). The frequency of NK cells seems unmodified in the blood of AD patients (43, 44). Recently, it was described that NK cells from these patients had increased expression of the receptor 5-HT2C for the neurotransmitter serotonin (45), which was shown to be implicated in either increase or decrease of NKCA (46, 47). However, patients...
Multiple sclerosis. Multiple sclerosis (MS) is the most common disabling neurologic disease in young adults. MS is a neuroinflammatory autoimmune disease affecting the CNS, characterized by widespread inflammation with infiltration of immune cells and demyelination. The involvement of NK cells in the pathogenesis of MS has been shown by several approaches: MS relapses were correlated with a deficit of NK cells, which were proposed to modulate the autoimmune response and to participate in the repair of neurologic damage (53); the functional activity of NK cells decreases during active phases of the disease and is restored during remission (54); and the treatment of MS patients with IFN-β (55) or daclizumab (56) results in decreased disease flares accompanied by a growing population of regulatory CD56bright NK cells. Moreover, during pregnancy, MS activity is reduced and the regulatory NK cell population is increased in blood (57). Interestingly, a reduction of blood NK cells expressing CX3CR1 correlated with disease severity (54). Because CD56bright NK cells were shown to be CX3CR1− in normal donors, it would be interesting to further look at the capacity of daclizumab to modulate CX3CR1 expression on NK cell subtypes (58). Manipulation of NK cells in animal models of EAE leads to contradictory results. According to some authors, NK cells play a deleterious role (15) during myelin oligodendrocyte glycoprotein EAE in C57BL/6 mice. On the contrary, numerous studies attribute, using the same EAE model, a neuroprotective role to NK cells. Depletion of NK cells, by injecting anti-NK1.1 or anti–asialo GM1 Abs, induced worsening of the disease, associated with increased proliferation of CD4+ Th17 cells and high production of cytokines (14). Overall, this suggests that NK cells are a potential target for future MS therapy.

Acute stroke and trauma. Acute CNS diseases, such as stroke and traumatic brain injury (TBI), have been associated with increased risk of infectious complications (59). It is thought that CNS injuries break the delicate interplay between the immune system and CNS, resulting in secondary immunosuppression (60). Many studies have shown a reduced NKCA of blood NK cells of patients with spinal cord injury and TBI (61–63). Although the CD56bright population stayed unchanged, the CD56dim subtype was decreased, and this correlated with the occurrence of infections after TBI (64). Alternatively, in the context of stroke, the NK cell counts showed a nonsignificant decrease (65–67). Nonetheless, there is a decrease of NK cells expressing IFN-γ and perforin, consistent with the reduction of their NKCA in the acute phase of ischemic stroke. Interestingly, others reported that deficiency in perforin expression was negatively correlated with Fas ligand expression by NK cells in individual patients (67). The decrease in IFN-γ secretion was also observed in a mouse model of middle cerebral artery occlusion leading to spontaneous septicaemia and pneumonia (68). The adoptive transfer of NK cells diminished bacterial load in the lung. These authors also showed evidence that the sympathetic nervous system participates in the immunosuppression induced by middle cerebral artery occlusion, as its blockage by a β2-adrenergic receptor antagonist prevents the defective IFN-γ response and the occurrence of bacterial infections.

Mental disorders. A growing body of research has found that mental disorders may influence somatic health and disease by altering immunity. During acute stress, such as public speech, there is a rapid mobilization of NK cells, with a CD56(dim) CD16−CD62L− phenotype, from various reservoirs into the blood (69). Acute stress correlates with increased NKCA (70). The NK cell redistribution was proposed to be coordinated via secretion of catecholamines from the sympathetic nervous system into the blood and further NK cell mobilization regulated through β2-adrenergic receptors (71). In contrast, in the context of chronic stress or depression, NKCA is markedly reduced compared with controls (72). Diminished NKCA was associated with a reduction of the CD16+ NK cell subset in blood of depressed patients (73). Additionally, several studies have found that antidepressants that block the reuptake of serotonin are associated with upregulation of NK cell functions in depressed individuals (74). The mechanism by which mental dysfunction acts on NK cells is still debated. Many hypotheses are proposed, including the deregulation of the hypothalamic/pituitary/adrenal axis leading to elevated levels of blood glucocorticoids, which further inhibit NKCA through epigenetic processes (75). Several other mental disorders are correlated with a diminution of NK cell functions, such as obsessive-compulsive disorder (76) and autism (77). In the latter study, the authors proposed that NK cell impairment in early life could impede neurogenesis, resulting in subsequent mental dysfunction, especially because neuronal stem cells were shown to express NKG2D ligands (78).

Phenotype and physiology of brain NK cells in health and disease

Healthy brain. NK cells have been found in most organs, where they present various phenotypes and functions related to the organ-specific environment (18). Although they could be recruited to the brain in response to several CNS disorders, there are sparse data about the phenotype of NK cells in the nascent CNS. In the C57BL/6 mouse model, NK1.1+ αβ TCR− NK cells represent 9.74% of total lymphoid cells within the brain before infection with P. berghei (38). We confirmed the presence of NK1.1+NKp46+ NK cells in the brain of C57BL/6 mice where they represented 11.1 ± 0.40% (n = 3) of CD45high cells (Fig. 1D). These NK cells were in the majority immature CD11blowCD27+ and CD11bhigh CD27+ cells and in the minority mature CD11bhighCD27− cells, representing 58.9 ± 4.3, 27.0 ± 3.9, and 5.7 ± 1.3%, respectively (Fig. 1E). This profile corresponds to the one in LNs (Fig. 1F), which contrasts with a higher percentage of mature NK cells in spleen or blood (Fig. 1G, 1H).
cells, and the brain appears as a “new” compartment for future investigation of their trafficking. In steady-state conditions, CD4+ T cells were found to patrol from the blood through the choroid plexus into the CSF to perform CNS immunosurveillance (1). Thus it is conceivable that NK cells follow the same route to scan the CNS milieu for pathological components either directly through their surface receptors or by interaction with brain-resident immune cells (Fig. 3). NK cell subsets express a different array of chemokine receptors in humans and mice. For example, human CD56+ NK cells were shown to express high levels of CCR7 and CXCR3, so they can migrate in response to CCL19 and CXCL10, which are constitutively expressed in the CNS (81). Moreover, TGF-β is constitutively present in the CSF and was found to modulate macrophages (82). Because TGF-β was recently shown to negatively regulate maturation of NK cells, it might be relevant to investigate ex vivo whether particular CSF constituents might modify NK cell phenotypes (83). The reason for the presence of immature NK cells in the healthy CNS needs further experiments to delineate whether the brain influx of NK cells is dependent on their phenotype or whether it is the CNS milieu that further influences such influx.

CNS disorders. Although the possible immune surveillance of the CNS by NK cells is still enigmatic, their infiltration following brain pathology is more characterized. Nonetheless, their mode of entry into CNS and the molecules leading to this recruitment are poorly understood. This recruitment could be coordinated by CNS resident cells, such as microglia, astrocytes, and neurons, that secrete chemokines involved in NK cell migration, such as CCL2, CXCL10, and CX3CL1 (3, 18, 38).

In intracranial tumors, NK cells account for a minor part of infiltrating leukocytes as shown in GBM, pilocytic astrocytomas, and brain metastases of carcinoma (2, 84–86). According to our observations, NK cells comprise 2.11 ± 0.54% of infiltrating immune cells in GBM (J. Kmiecik, A. Poli, N.H.C. Brons, A. Waha, G.E. Eide, P.O. Enger, J. Zimmer, and M. Chekenya, unpublished observations). These tumor-infiltrating NK cells were shown to be inhibited by glioma-derived factors but also by other local immune cells with anti-inflammatory properties (20). Nonetheless, immu-

FIGURE 1. Distribution of NK cell subsets in B6 mice. Tissues were disrupted using dissociation buffer containing benzonase (except whole blood); brain cells were further enriched by Percoll density gradient. Cell suspensions were stained for NK1.1, NKp46, CD45, CD27, CD11b, and live/dead. First, (A) dead cells and (B) doublets were excluded, (C) immune cells (CD45+) were gated, and then (D) NK cells were gated as NK1.1-NKp46+ events. Representative dot plots show the profiles of CD27 and CD11b expression of NK cells in (E) brain, (F) LNs, (G) spleen, and (H) whole blood.

We demonstrated the presence of immature NK cells in the brain of healthy mice. We excluded a contamination by blood NK cells, as animals were extensively perfused with PBS and because they display different phenotypes. To our knowledge, there is no evidence for the presence of NK cells in non-pathological human brain (NPB) parenchyma because of obvious ethical considerations. Nonetheless, we observed rare NK cells in the CSF of patients with either peripheral neuropathy or MS, exhibiting mainly CD56+ phenotypes (Fig. 2A). This repartition is typical of the phenotype of NK cells found in LNs and is different from blood NK cells (Ref. 10 and Fig. 2B). Recently, Hamann et al. (79) observed an overall diminished frequency of NK cells in CSF compared with blood with a CSF/blood ratio of 1:4.

The validity of comparing human and mouse NK cell subsets and their maturation is still debated, despite that CD27+ mouse NK cells show some functional and phenotypic similarity to the CD56+ human subset such as the receptor expression pattern. However, the CD27+ mouse subset is highly cytotoxic as opposed to the CD56+ human NK cells (8). Nonetheless, it has been proposed that human NK cell maturation could be further delineated according to the expression of CD11b and CD27 (80), such as in the mouse. The same team demonstrated that CD56+ NK cells are mainly CD27-, indicating that human brain NK cells may display an immature phenotype, although a deeper analysis is needed to confirm this.

NK cells are known to actively screen nonlymphoid and lymphoid organs in search of infected targets and transformed

FIGURE 2. Lymphocyte subsets in paired (A, C) CSF and (B, D) blood samples of representative patients with NPB (A, B) and with MS (C, D). NK cells were gated as CD45+CD3+CD56+ and CD56+. Numbers represent the percentage of cells among the CD45+ population.

FIGURE 3. CNS NK cell physiology could be modulated by brain-resident immune cells following CNS compartments. The passage of NK cells from body vasculature through the CSF by the BCSFB at the choroid plexus may be privileged at steady-states. Nonetheless, they may also cross the BBB when circulating in brain vessels to enter the meninges, perivascular space, and brain parenchyma.
notherapies are emerging to increase this NK cell recruitment and function. As an example, oncolytic HSV infection of GBM in mice resulted in a massive influx of NK cells. Interestingly, although NK cells recruited to tumors in control mice were mostly CD11b<sup>high</sup>CD27<sup>low</sup>, oncolytic HSV administration resulted in recruitment of CD11b<sup>high</sup>/low CD27<sup>+</sup> NK cells (87). This subpopulation was able to activate macrophages/microglia through secretion of IFN-γ. In a rat xenograph model of GBM, we also observed an increase of proinflammatory macrophages/microglia following intracranial injection of activated NK cells leading to beneficial outcome (A. Poli, J. Wang, O. Domingues, C.B Rygh, T. Yan, F. Thorsen, J. Planagumà, E. McCormack, F. Hentges, P.H.Pedersen, J. Zimmer, P.O. Enger, and M. Chekenya, submitted for publication). Thus, brain NK cells may influence the disease outcome by interaction with other CNS immune cells.

Brain NK cells were observed in greater abundance following CNS infection (6, 38). However, NK cell recruitment could be either beneficial or detrimental depending on the infectious agent. Thus, although the recruitment of NK cells was shown to be essential for controlling mouse hepatitis virus and Semliki Forest virus replication within the CNS through an IFN-γ-dependent mechanism (3, 4), their cytolytic activity (Fas and granule exocytosis dependent) was shown to potentiate the immunopathology, such as increased animal death and brain demyelination (Fig. 4) (4). Alternatively, NK cells were shown to be essential for controlling parasitism; however, their depletion protects C57BL/6 mice against P. berghei–mediated cerebral malaria. It seems that NK cells stimulated DCs to produce IL-12 required for T cell priming, leading to subsequent cerebral disease (Fig. 4) (38, 39). In neuroborreliosis, NK cell numbers in the CSF are significantly higher than in tick-borne viral encephalitis, in correlation with the level of IFN-γ found in CSF (88). The outcome of CNS infections varies according to the type of pathogens, and it is also dependent on interindividual genetic and environmental factors. The immune response following infection can contribute to fatal disease, but is also necessary for recovery, and the specific role of immune cells, including NK cells, is not well described (89). Furthermore, many teams propose that neurodegenerative diseases could be a consequence of CNS infections (89). Thus, repeated infection by HSV was proposed to be at the origin of AD and MS emergence, as pathological features of this CNS infection share similarity with neurodegenerative diseases, such as neuronal death (89). Moreover, several models of CNS infection in mice were associated with parenchymal demyelination (4). The recruitment of immune effectors, including NK cells, following infection leads to the release of cytotoxic agents, such as granzymes, perforin, and proinflammatory molecules, which could generate direct or indirect cellular damages leading to neurodegenerative foci (89) (Fig. 4). As a comparable example, liver NK cells were implicated in the emergence of fulminant hepatic failure following mouse hepatitis virus infection, whereas the depletion of NK cells limited liver damage and increased animal survival (90). Alternatively, IFN-γ was also associated with both neuronal protection and repair by modulation of microglia properties (91), so we can propose that NK cells secreting IFN-γ following infection may have a neuroprotective function (Fig. 4). Furthermore, NK cells were shown to secrete IL-10 following viral infection of the liver, and it could be interesting to investigate this in the context of CNS infection and neuroprotection (92). All of these facts indicate why NK cells could be implicated in neurodegeneration and/or neuroprotection (Fig. 4).

During the last decade, the major increase in knowledge about CNS NK cells arose from studies of MS patients and animal models of EAE. The EAE model revealed that NK cell homing to the CNS is essential to control neuroinflammation (Fig. 4). Indeed, recruited NK cells were shown to kill microglia responsible for the priming of autoreactive T cells.

**FIGURE 4.** NK cells could initiate neurotoxicity and neuroprotection following brain disorders. NK cells diminished animal survival in cerebral malaria by activation of cytotoxic T cells and DCs through secretion of IFN-γ. The exocytosis of cytotoxic granules following CNS infection may also initiate inflammatory cascades leading to brain injury. Alternatively, IFN-γ could generate neuroprotection through activation of microglia with neuroprotective function. Finally, it was described that in the context of EAE, NK cells reduce neurotoxicity of autoreactive T cells by killing proinflammatory microglia.
References
10. Nimmer, et al. (95) found that the influx of CD3−CD161bright NK cells in experimental traumatic brain contusion in DA rats was more marked than in PVG rats. This interindividual genetic heterogeneity might influence the outcome and treatment response following CNS pathology and partially explain the differences observed between studies.

Conclusions
In this review we have discussed the systemic NK cell phenotype and functional alterations involved in the most common brain disorders. Note that the characterization of NK cells was often not well performed, as many studies used uninformative or heterogeneous NK cell populations. Differential responses were mainly observed in cohorts of patients with suspected MS described an increased absolute NK cell number in the CSF compared with patients with other neurologic defects (unpublished data). These NK cells display an immature phenotype with high CD56 expression in a comparison with kidney and bladder cancer patients. Particular features of cell-mediated immunity in patients with anaplastic gliomas: a comparison with kidney and bladder cancer patients. J. Immunol. 180: 4495–4506.


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