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Review article

# The role of natural killer cells in curbing neuroinflammation

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**Abstract**

Natural killer (NK) cells are evolutionarily early lymphocytes that lack antigen-specific receptors and, hence, are considered to be part of the innate immune system. The majority of research on NK cells has focused on their ability to lyse "target cells", generally identified by low or absent MHC Class I expression, such as tumor cells and virus infected cells. However, an alternative role of these leukocytes as regulators of adaptive (and potentially destructive) immune responses, in particular organ-specific autoimmune diseases, has been increasingly recognized. Here we discuss the growing body of evidence that NK cells limit damage in autoimmune demyelinating disease by inhibiting autoreactive T cell responses without harming resident neurons or glia.


*Keywords:* Multiple sclerosis; Experimental autoimmune encephalomyelitis; Natural killer cells; Immunoregulation

**Contents**



## 1. Introduction

Over the past few years the majority of studies on immunoregulatory networks have focused on the role of thymic derived and inducible FoxP3$^+$ CD4$^+$ T cells, IL-10 producing Tr1 cells and TGF-β producing Th3 cells (Baecher-Allan and Hafler, 2006; Carrier et al., 2007; Miyara and Sakaguchi, 2007; Roncarolo et al., 2006). This trend may create the impression that the adaptive arm of the immune system has primary responsibility for monitoring its own activities. However, multiple studies in the literature provide evidence that cells within the innate immune system have the potential to inhibit autoreactive CD4$^+$ T cells from mediating autoimmune disease and foreign antigen reactive CD4$^+$ T cells from inflicting collateral damage to healthy tissues. In addition to so called "suppressor" myeloid cells (Nagaraj and Gabrilovich, 2007; Serafini et al., 2006) and resting or "homeostatic" CD205$^+$ dendritic cells (Hawiger et al., 2001), NK cells are emerging as key participants in the immunomodulatory circuitry.

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Natural killer (NK) cells are evolutionarily primitive lymphocytes that possess cytotoxic properties, classically directed against transformed and virus infected cells. Unlike T and B cells, NK cells are not antigen specific. Their cytotoxicity is determined by the collective signaling of an array of inhibitory and stimulatory receptors expressed on their surface (Borrego et al., 2006; Kirwan and Burshtyn, 2007). NK cell inhibitory receptors, commonly referred to as killer inhibitory receptors or KIRs, interact with classical and non-classical MHC Class I molecules. Hence, the presence of self MHC Class I molecules on healthy cells sends an inactivating signal to NK cells. Examples of KIRs include the Ly49 family of molecules (that bind classical MHC Class I) and NGK2A (that binds Qa-1, a non-classical MHC Class I molecule expressed on activated T cells, B cells and dendritic cells). Conversely, NK cell cytotoxicity is generally elicited by a distinct set of inducible molecules that have a weak homology with MHC Class I and bind NK stimulatory receptors. For example, retinoic acid early inducible gene (RAE-1) encoded proteins, distantly related to MHC Class I, are ligands for the murine NK cell stimulatory receptor, NGK2D (Diefenbach et al., 2000; Smyth et al., 2005). In many instances, RAE-1 expression correlates with susceptibility to NK cell mediated cytotoxicity (Backstrom et al., 2003). RAE-1 was originally isolated from tumor cell lines (Nomura et al., 1994); it is also expressed on virus infected cells (Backstrom et al., 2007).

Once activated, NK cells mediate cytotoxicity via contact-dependent pathways involving perforin/granzyme, Fas/Fas-ligand and TRAIL/TRAIL ligand interactions (Screpanti et al., 2005; Takeda et al., 2001; Warren and Smyth, 1999). They also produce pro-inflammatory cytokines, such as IFNγ, and chemokines such as CCL5 (Biron et al., 1999). A premise of this review article is that, while NK cells originally evolved as a defense against microbes and neoplasms, their cytotoxic properties were later subverted to eliminate potentially harmful T cell responses. Here we will limit our discussion to the putative regulatory role of NK cells in EAE and MS.

A number of laboratories have found an inverse relationship between the frequency or functional competence of circulating NK cells and clinical or radiological disease activity in patients with multiple sclerosis (MS) (Kastrukoff et al., 2003; Kastrukoff et al., 1998; Oger et al., 1988). In at least two publications the administration of immunomodulatory drugs to MS patients was associated with an expansion of circulating NK cells (Bielekova et al., 2006; Saraste et al., 2007). Furthermore, a substantive body of research demonstrates that conventional ($CD3^{-}TCR^{-}NK1.1^{+}$) NK cells limit the severity of experimental autoimmune encephalomyelitis across several different models (Galazka et al., 2006; Huang et al., 2006; Lu et al., 2007; Matsumoto et al., 1998; Xu et al., 2005; Zhang et al., 1997). Collectively this data suggest a significant role of NK cells in reducing neuroinflammation and CNS injury in the context of autoimmune demyelinating disease. Topics that remain to be addressed include: the mechanism and site of action of regulatory NK cells in EAE, factors that influence the migration and biological activities of regulatory NK cells *in vivo*, effects of NK cells on CNS resident neurons and glia, and the feasibility of using NK cells as therapeutic targets and/or markers of CNS disease activity in MS.

## 2. Natural killer cells modulate the clinical severity of EAE

In 1997 Zhang et al. reported that treatment of $MOG_{35–55}$-sensitized C57BL/6 mice with a depleting antibody specific for NK1.1 accelerated the onset and increased the severity of clinical EAE (Zhang et al., 1997). In addition, administration of anti-NK1.1 converted an ordinarily monophasic clinical course into a relapsing one. In parallel experiments, MOG-specific $CD4^{+}$ T cell lines induced a more severe form of EAE in syngeneic hosts treated with an anti-NK1.1 antibody than in hosts treated with an isotype matched control antibody. Similar observations were made using C57BL/6 β2-microglobulin deficient mice that lack $NK1.1^{+}$ $CD3^{+}$ cells, indicating that depletion of conventional NK cells was responsible for the therapeutic effect of the antibody. (This point is particularly important since it has been speculated that NK T cells play a regulatory role in autoimmune diseases, including EAE and MS (Furlan et al., 2003; Illes et al., 2004)). Conversely, cotransfer of whole splenocytes, but not NK cell depleted splenocytes, ameliorated EAE induced by the injection of myelin-specific T cells into Rag2−/− hosts.

Shortly thereafter, Matsumoto et al. published their finding that injection of MBP-immunized rats with antibodies specific for either NKR-P1 (analogous to NK1.1) or asialo GM1 resulted in aggravation of EAE, reflected by significantly higher maximal clinical scores (and, with anti-asialo GM1, increased mortality rates) (Matsumoto et al., 1998). However, these experiments were not designed to distinguish between effects of the antibodies on NK cells as opposed to NK T cells (with regard to both anti-NKR-P1 and anti-asialo GM1) or peritoneal macrophages and $CD8^{+}$ T cells (with regard to anti-asialo GM1) .

While the manuscripts referenced above do not elucidate the mechanism of action of regulatory NK cells in EAE, they provide some clues. Hence, as mentioned earlier, Zhang et al. reported that NK cell depletion exacerbates adoptively transferred EAE. Masumoto et al. found that NK cells comprise up to 17% of CNS-infiltrating inflammatory cells in Lewis rats at peak disease. Although the study by Zhang et al. did not include flow cytometric analysis of CNS mononuclear cells, other researchers have reported that NK cells account for 10–20% of the infiltrate in symptomatic C57BL/6 mice immunized with $MOG_{35–55}$ (Huang et al., 2006). Collectively, the observations that significant numbers of NK cells accumulate within the target organ during EAE, and NK depleting antibodies aggravate disease at a point past the priming of encephalitogenic T cells, implicate a role of regulatory NK cells in the effector phase of pathogenesis, possibly within the CNS itself. On the other hand, lymph node cells from anti-NK1.1 treated, $MOG_{35–55}$ immunized C5LBL/6 mice mount enhanced MOG-specific proliferation and IFNγ recall responses, suggesting that NK cells ordinarily suppress the priming, differentation and/or expansion of encephalitogenic T cells in the periphery (Zhang et al., 1997). Of course, it is possible that the immunomodulary activities of NK cells are important during both induction and effector stages. In fact, reigning theories on their mechanism of action (namely that they drive activation induced cell death and directly lyse encephalitogenic cells) are compatible with such a scenario.