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Human NK Cells Kill Resting but Not Activated Microglia via NKG2D- and NKp46-Mediated Recognition

Anna Lüinemann,* Jan D. Lüinemann,* Susanne Roberts,* Brady Messmer,* Rosa Barreira da Silva,* Cedric S. Raine,† and Christian Münz2*

Microglia are resident macrophage-like APCs of the CNS. To avoid escalation of inflammatory processes and bystander damage within the CNS, microglia-driven inflammatory responses need to be tightly regulated and both spatially and temporally restricted. Following traumatic, infectious, and autoimmune-mediated brain injury, NK cells have been found in the CNS, but the functional significance of NK cell recruitment and their mechanisms of action during brain inflammation are not well understood. In this study, we investigated whether and by which mechanisms human NK cells might edit resting and activated human microglial cells via killing in vitro. IL-2-activated NK cells efficiently killed both resting allogeneic and autologous microglia in a cell-contact-dependent manner. Activated NK cells rapidly formed synapses with human microglial cells in which perforin had been polarized to the cellular interface. Ab-mediated NKG2D and NKp46 blockade completely prevented the killing of human microglia by activated NK cells. Up-regulation of MHC class I surface expression by TLR4 stimulation protected microglia from NK cell-mediated killing, whereas MHC class I blockade enhanced cytotoxic NK cell activity. These data suggest that brain-infiltrating NK cells might restrict innate and adaptive immune responses within the human CNS via elimination of resting microglia.


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Listener's Note

NK cells have been suggested to edit human myeloid cells during immune responses via cytotoxic activity (6, 7). This NK cell effector function is controlled by germline-encoded activating and inhibitory receptors on these innate lymphocytes. Only if more activating signals are received due to high expression of activating NK cell receptor ligands or loss of inhibitory NK cell receptor ligands, NK cell killing is unleashed. The main activating NK cell receptors are NKG2D, the natural cytotoxicity receptors NKp30, NKp44, and NKp46 and DNAM-1. The ligands for these activating receptors are primarily up-regulated upon cellular stress, like during viral infections or transformation (8, 9). As inhibitory structures, MHC class I molecules are primarily recognized by killer Ig-like receptors or CD94/NKG2A (8, 9). Therefore, stressed cells with down-regulated MHC class I molecules are preferentially targeted by NK cells.

The immune privilege of the CNS is thought to be maintained by endothelial tight junctions of the blood-brain barrier (BBB) and the presence of an immunosuppressive microenvironment (10). BBB disruption leads to recruitment of a large number of leukocytes into the CNS, among which NK cells have been shown to accumulate in the CNS following traumatic peripheral axotomy (11–13) and in the course of infectious as well as autoimmune CNS inflammation (14–17). Whereas the frequency of CNS-infiltrating NK cells in acute and chronic neuroinflammatory diseases in humans has so far not been thoroughly characterized, it was estimated that NK cells comprise 10–20% of CNS infiltrates in experimental autoimmune encephalomyelitis (EAE) (18) models (15, 16). However, the functional significance of NK cell recruitment and their mechanism of action during brain inflammation are not well understood. To address a possible immunoregulatory function of NK cells within the inflamed human CNS, we investigated NK cell interactions with resting and activated human MG.

Materials and Methods

Primary human microglia cell culture

Second trimester fetal brain tissue was obtained under institutional review board-approved protocols from the fetal tissue repository at the Albert Einstein College of Medicine (New York, NY) or ABR (Alameda, CA).
Mixed human fetal CNS cell cultures were prepared by enzymatic and mechanical dissociation of the cerebral tissues followed by filtration through nylon meshes of 250- and 130-μm pore sizes, as described previously (18). Cells were plated at 10^6–10^7 cells/ml in DMEM (supplemented with 4.5 g/L glucose, 4 mM t-glutamine, and 25 mM HEPES; tryptophan concentration, 16 μg/ml; Cellgro) plus 5% FCS (Gemini Bio-Products), penicillin (100 U/ml), streptomycin (100 μg/ml), and amphotericin B (0.25 μg/ml; Fungizone) for 2 wk. Briefly, highly enriched cultures of human fetal MG (>98% CD11b+ cells as determined by flow cytometry, compared with respective isotype control) were generated by gentle collection of floating cells in confluent monolayer cultures consisting of astrocytes, neurons, and MG at 2–3 wk in vitro. Cells were seeded in low cell-binding 24-well-plates (Nunc) at 250,000 cells/well. In some experiments, microglia were preactivated for 48 h with 100 or 200 ng/ml LPS from *Salmonella minnesota* (Sigma-Aldrich).

**Isolation of NK cells**

NK cells were isolated by negative selection using the NK cell Isolation Kit II (Miltenyi Biotec) according to the manufacturer’s instructions. The purity of the isolated B cells and NK cells was higher than 90% and contained <5% contaminating T cells as determined by flow cytometry. Separated NK cells were activated with 100 U/ml IL-2 for 5 days before coculture. IL-2 was used as a classical NK cell mitogen, which could be produced by activated T cells which constitute a major component of inflammatory CNS infiltrates (21).

**IFN-γ production assay**

For intracellular staining of IFN-γ, activated NK cells were incubated with PKH26-labeled MG at a ratio of 10:1 at 37°C in RPMI 1640 plus 5% human serum plus gentamicin. Brefeldin A was added after 1 h of coculture and an additional 5 h later cells were harvested and stained with Aqua Live/Dead Cell stain (Invitrogen) according to the manufacturer’s protocol. After fixing the cells with 2% paraformaldehyde, they were permeabilized and stained with fluorochrome-labeled anti-IFN-γ. PKH26+, live, small cells were analyzed using FlowJo software (Tree Star).

**Flow cytometry analysis**

Microglia were stained with fluorochrome-labeled Abs specific for CD11b (M1/70; BD Biosciences), MHC class I HLA-A,B,C (W6/32; BioLegend), poliovirus receptor (CD155; MBL), and their respective isotype controls for 30 min at 4°C. The unlabeled Nectin-2-specific Ab (Santa Cruz Biotechnology) was followed by an incubation with a fluorochrome-labeled secondary anti-mouse IgG Ab (BD Biosciences) for 30 min at 4°C. For NG2G2 dendritic staining, biotinylated NG2G2 monomers were provided by A. Steinle (University of Tübingen, Tübingen, Germany) and tetramerized as previously described for MHC class I tetramers (22). HLA-A2/HIV gag peptide tetramers served as a control for NG2G2 tetramer staining of NG2G2 dendritic cells. After two washes, cells were resuspended in 200 μl of PBS. At least 30,000 events were collected on a BD Biosciences LSR II flow cytometer. Frequencies were calculated using FlowJo software (Tree Star).

**TO-PRO3 iodide (TP3) cytotoxicity assay**

Cytotoxicity of NK cells was quantified using the previously described flow cytometry-based TP3 (Invitrogen) assay (23). It has to be noted that this assay might underestimate the total number of dead cells, since the frequency of dying cells in this assay is based on the forward scatter/side scatter live cell gate rather than on release of chromium 51. A number of previous studies have, however, shown that cytotoxicity measured by the flow cytometric cell-mediated cytotoxicity assay correlates well with the conventional chromium 51 release assay and the PKH-26/TP3-based assay is now widely used as a reproducible experimental system for NK cell and Ab-dependent cell-mediated cytotoxicity (23–26). Briefly, human microglial cells, macrophages, or DCs were labeled with a membrane dye, PKH-26, to allow discrimination of target cells after incubation with effector cells. Postincubation, cell death within the PKH-26+ target cell population was assessed by the addition of the viability probe TP3 (Invitrogen). Flow cytometry data were acquired on a BD Biosciences LSRII flow cytometer and analyzed using FlowJo software (Tree Star). This method allows analysis to be conducted on a single-cell basis and overcomes the need for radioactive chemicals. For neutralization experiments, NK cells were preincubated for 1 h at 37°C with a 10 μg/ml Ab concentration. The blocking Abs, NG2G2 (BATT211), NKp30 (F252), and NKp46 (KL247) were gifts from Drs. A. Moretta (University of Genoa, Genoa, Italy) and Guido

**Figure 1.** NK cells kill human MG. Fetal human microglial cells were cocultured with allogeneic (upper panel) or autologous (lower panel) IL-2-activated NK cells for 4 h. The extent of cytotoxicity was quantified by the relative number of live human MG cells labeled with PKH-26 and dead, permeabilized cells labeled with both PKH-26 and TP3. A and E, Spontaneous permeabilization of primary human MG. B, C, F, and G, Permeabilization at different E:T ratios. D and H, Diagrams summarize at least three experiments and display means and SEM of TP3+ MG cells compared with PKH26+ MG cells after coculture with increasing E:T ratios. D, MG vs 10:1: ***p < 0.0001 for E:T ratio of 10:1. H, MG vs 10:1: *p = 0.0492 for E:T ratio of 10:1.
FIGURE 2. NK cells produce IFN-γ upon recognition of MG. IL-2-activated NK cells were cocultured with MG in an E:T ratio of 10:1, as for the cytotoxicity assay. Intracellular staining for IFN-γ was performed after 6 h of coculture. The frequency of IFN-γ-producing activated NK cells was 6.5-fold higher following MG compared with nontarget cell recognition (mean frequency = 0.31 ± 0.05 vs 0.05 ± 0.02; p = 0.02). Representative and summarized data of four experiments are shown.

Ferlazzo (University of Messina, Messina, Italy). In addition, MHC class I Ab (W6/32; BioLegend), DNAM-1 (mAb 666; R&D Systems), LFA-1 (CD11a; BioLegend), and 2B4 (CD244; Beckman Coulter) were used. Expression of ligands for the latter two molecules (ICAM-1 and CD48, respectively) by microglial cells could previously be demonstrated (27–29).

Conjugation of human MG with NK cells

For conjugation assays, resting or IL-2-activated NK cells were cocultured with MG. Cells were mixed at a NK cell:MG ratio of 1:2 in 20 μl of RPMI 1640 without serum before being pelleted at 10,000 g. The pellets were allowed to conjugate for 1 min at 37°C. After initial conjugation, cells were gently resuspended and centrifuged onto poly-lysine-coated 1.5-mm coverslips for immunofluorescence analysis. Cells on slides were fixed in 3% paraformaldehyde for 20 min at 4°C. Cells were permeabilized with 0.01% Triton X-100 for 1 min at room temperature and stained with anti-talin (TD77) or anti-perforin (6G9) Abs (Chemicon International), followed by Alexa Fluor 555-conjugated rabbit anti-mouse IgG Ab (Invitrogen). Control Abs used were mouse IgG1 (MOPC-21; BioLegend) and mouse IgG2b,κ (MOPC-141; Sigma-Aldrich). All washes were performed in PBS supplemented with 1% fish skin gelatin (Sigma-Aldrich) and 0.02% saponin (Sigma-Aldrich). Slides were counterstained with 4,6-diamidino-2-phenylindole and mounted with Prolong gold antifade reagent (Invitrogen) and visualized with an inverted Olympus IX-70 microscope (DeltaVision Microscope; Applied Precision/Olympus) and a Photometrics CoolSnap QE camera. Serial optical sections (0.2 μm; 20 sections) were acquired for all labelings. Images were then deconvoluted using DeltaVision SoftWoRx software version 3.4.4, and photographs were overlaid using Metamorph software version 6.3.6 (Universal Imaging). Molecules at the synapse were quantified with ImageJ software. The enrichment of talin at the contact site of each conjugate was measured and compared with the distribution of the same molecule in a similar area opposite to the contact site within the same cell. The relative enrichment (RE) was calculated as the average intensity per volume unit at the contact site or in an equivalent volume opposite to the contact site divided by the average intensity per unit volume of the entire cell. The area of the contact site was defined manually and represented 16.8 ± 4.2% of the total cell volume. The area across the contact site was defined manually and represented 17.2 ± 4.4% of the total cell volume. The values obtained in unconjugated cells were considered as baseline, and data represent the ratio of RE at the contact site to RE at an equivalent volume opposite from the contact site normalized to the values in single cells, assigned as 100%. The percentage of conjugates showing perforin polarization was assessed in randomly selected fields after the analysis of at least 500 conjugates per experiment.

Statistics

Statistical analyses were performed with the Student t test using GraphPad Prism software. A p < 0.05 was considered significant. Plotted data are displayed as mean with SEM.

Results

Activated NK cells kill allogeneic and autologous human MG in a cell contact-dependent manner

To determine whether human microglial cells are susceptible to NK cell-mediated cell editing by killing, we performed a flow

FIGURE 3. Synapse formation and perforin polarization in NK cell-MG conjugates. Polarization of the cytoskeletal protein talin, indicative of synapse formation, and of the cytotoxic molecule perforin in NK cells was determined by two-dimensional immunofluorescence microscopy after 1 min. A, Perforin and talin polarization in IL-2-activated NK cells conjugated to MG. B, Perforin is polarized in conjugates of MG with IL-2-activated NK cells. Nonpolarized vs polarized perforin in conjugates with resting NK cells: **, p = 0.001; nonpolarized vs polarized perforin in conjugates with activated NK cells: ***, p < 0.0001. C, RE of talin to cell contact areas in NK cell conjugates with MG compared with NK cells alone. Resting NK cells alone vs conjugated: ***, p < 0.0001; activated NK cells alone vs conjugated: ***, p < 0.0001; Student’s t test. Representative and summarized data for three experiments are shown. DIC, Differential interference contrast.
cytometry-based cytotoxicity assay using the fluorophores PKH-26 to label MG and the DNA intercalating dye TP3 to determine the viability of PHK-26/H11001 target cells. The extent of cytotoxicity was quantified by the relative number of live human microglial cells labeled with PKH-26 and dead, permeabilized cells labeled with both PKH-26 and TP3 (23). As shown in Fig. 1, IL-2-activated NK cells efficiently killed microglial cells at E:T ratios of 10:1 after 4 h of incubation. Although the relative contribution of microglial cells and NK cells to the total cell number within CNS lesions associated with BBB disruption has so far not been characterized, we propose that such an E:T ratio might be specifically relevant during acute CNS inflammation during which NK cells comprise 10–20% of CNS cellular infiltrates (15, 16). In addition, MG cell recognition was associated with induction of IFN-γ production (Fig. 2) by activated NK cells, indicating that MG triggers both NK cell cytokine production and cytotoxic activity. MG cells, cocultured with autologous NK cells isolated from fetal liver tissue, were killed at similar levels as allogeneic target cells (Fig. 1), suggesting that the MHC class I polymorphism at the low constitutive expression levels on microglial cells modifies NK cell killing only to a low extent. To assess the dependency on soluble factors as opposed to direct cell-cell contact on microglial cell cytotoxicity, we next quantified NK cell cytotoxic activity in Transwell vs non-Transwell cocultures. As shown in Fig. 2, the frequency of TP3/H11001 MG cells in Transwell cultures at E:T ratios of 10:1 was similar to spontaneous microglial cytotoxicity, demonstrating that NK cell killing of human MG was primarily cell contact dependent. These results suggested that resting MG could be killed by activated NK cells.

### Rapid synapse formation and perforin polarization in NK cell-MG conjugates

To further analyze the cell contact dependency of MG killing by NK cells, we determined the formation and kinetics of cell-cell contact interactions between NK cells and microglial cells in vitro. For this purpose, we analyzed the polarization of the cytoskeletal protein talin, indicative of synapse formation, and of perforin, a pore-forming molecule of cytotoxic lymphocytes, in NK cells by two-dimensional immunofluorescence microscopy after 1 min. As shown in Fig. 3, talin was evenly distributed at the cell surface in resting MG cells, whereas perforin was newly synthesized and polarized at the site of conjugates. These results suggest that perforin is synthesized de novo upon conjugation with MG cells and is sequentially polarized at the site of contact.

### MHC class I blockade enhances NK cell cytotoxicity toward human MG

MG cells were cocultured with purified and IL-2-activated NK cells for 4 h in the presence and absence of a monoclonal-blocking MHC class I-specific Ab or an isotype control. Specific cell lysis was quantified by TP3 staining. The diagram displays means and SEM of TP3/H11001 MG cells compared with PKH26/H11001 MG cells after coculture. MHC class I vs IgG1: *p = 0.0171. Representative data for three experiments are shown.
unconjugated, resting, and activated NK cells. In MG/NK cell conjugates, talin was enriched at the cell contact area compared with the opposite side from the interface between MG and NK cells (RE mean ± SEM: 169.2 ± 4.5% in resting NK cells and 230.2 ± 5.9% in activated NK cells, respectively). The percentage of conjugates showing perforin polarization was assessed after the analysis of at least 500 conjugates per experiment in randomly selected fields. When resting NK cells formed conjugates with MG, 31.4 ± 4.7% (mean ± SEM) of conjugates recruited perforin to the contact area within 1 min of coculture (Fig. 3B) compared with 67.2 ± 2.7% (mean ± SEM) when activated NK cells formed conjugates with MG. These data indicated that NK cells rapidly formed immunological synapses with human microglial cells and that the cytotoxic molecule perforin was rapidly polarized to the interface between MG and NK cells.

NK cell-mediated cytotoxicity toward human MG is NKG2D- and NKp46-dependent and enhanced by MHC class I blockade

Because the NK-mediated killing of tumor as well as normal cells can utilize a number of activating NK cell receptors, we analyzed whether mAb-mediated masking of some of these receptors could affect NK cell cytotoxicity against microglial cells. As shown in Fig. 4, the addition of anti-NKp30 mAb as well as the isotype control Ab had no effect on cytotoxicity. In contrast, a marked inhibition was detected upon addition of anti-NKp46 mAb and NK-mediated cytotoxicity was virtually abrogated following blockade of the activating NKG2D receptor. DNAM-1-, LFA-1-, and 2B4-specific monoclonal-blocking Abs had no effect on NK cell-mediated lysis of human microglial cells (Fig. 4C). The observation that cell contact-dependent NK cell killing of MG is not inhibited by LFA-1 blockade indicates a certain level of redundancy of adhesion molecules in mediating synapse formation between the two cell types (30). Moreover, in contrast to immature DCs, which are killed via NKp30, NKp46, and DNAM-1-mediated recognition (31–33) but similar to macrophages which have been found to be susceptible to NKG2D-dependent cytotoxicity by NK cells (19), our data indicate that MG cell lysis by NK cells is specifically dependent on NKG2D- and NKp46-mediated recognition.

We also demonstrated that blockade of the MHC class I molecules, HLA-A,B,C, enhanced NK cell-mediated cell cytotoxicity, indicating that the modest MHC class I expression of MG, nevertheless, inhibited NK cell killing to some extent (Fig. 5). These data indicated that the opposing activating and inhibitory signals, involved in NK cell recognition of MG, were mediated by the

FIGURE 6. Surface expression of ligands for activating NK cell receptors, i.e., NKG2D and DNAM-1, on resting and activated microglial cells. NKG2D ligands were constitutively expressed on human microglial cells in vitro and down-regulated following LPS stimulation (p < 0.0005) (A). MG also constitutively expressed DNAM-1 ligands, poliovirus receptor (PVR; B) and Nectin-2 (C), whose expression remained unchanged following LPS stimulation. Summarized data from three experiments are shown.

FIGURE 7. TLR ligation on human MG by LPS protected microglial cells from NK cell-mediated cytotoxicity (A); *, p = 0.02. In contrast, monocyte-derived macrophages were more susceptible to NK cell-mediated cell lysis following LPS stimulation (C), ***, p = 0.004, whereas matured DCs were less susceptible (B), ***, p < 0.001. In addition, LPS stimulation resulted in an up-regulation of MHC class I surface expression on microglial cells and the protective effect of MG activation could be reversed by MHC class I blockade, *, p = 0.002, indicating that this effect was primarily MHC class I mediated (D). At least three experiments for each cell type are summarized.
activating NK cell receptors NKG2D and Nkp46 on one side and MHC class I-binding receptors on the other.

Activated MG are protected from NK cell-mediated cytotoxicity

MG are resident macrophage-like cells of the CNS and recognize the presence of pathogens in part via TLR that detect ligands on bacterial, viral, and fungal pathogens. Since TLR signaling in macrophages has been shown to induce NKG2D ligand expression (34) and since TLR-mediated activation of monocyte-derived macrophages is reported to be associated with an increased susceptibility toward NK cell-mediated cytotoxicity (19), we investigated whether TLR ligation interfered with the expression of ligands for activating NK cell receptors, i.e., NKG2D and DNAM-1, and the susceptibility of MG cells toward NK cell cytotoxicity.

Using tetrameric NKG2D, we found that NKG2D ligands are constitutively expressed on human microglial cells in vitro and were down-regulated following LPS stimulation (Fig. 6A). MG also constitutively expressed DNAM-1 ligands, i.e., poliovirus receptor and Nectin-2, whose expression remained, however, unchanged following LPS stimulation (Fig. 6, B and C).

TLR ligation on human MG by LPS protected microglial cells as well as matured monocyte-derived DCs from NK cell-mediated cytotoxicity (Fig. 7, A and B). In contrast, monocyte-derived macrophages were more susceptible to NK cell-mediated cell lysis following LPS stimulation (Fig. 7C), indicating a functional difference of resting and activated primary MG compared with monocyte-derived macrophages. LPS stimulation resulted in an up-regulation of MHC class I surface expression on microglial cells and the protective effect of MG activation could be reversed by MHC class I blockade, indicating that this effect was primarily MHC class I-mediated (Fig. 7D). Altogether, these data suggested that activated MG, in contrast to macrophages, were less susceptible to NK cell-mediated cytotoxicity and that activation of MG was associated with expression of more inhibitory than activating ligands for NK cell receptors.

Discussion

Our study has shown that human microglial cells were susceptible to allogeneic and autologous NK cell editing by cytotoxicity. NK cells rapidly formed synapses with human MG cells, in which the cytotoxic molecule perforin was polarized to the cell contact area. Killing of MG was dependent on NKG2D and Nkp46 engagement and counterbalanced by MHC class I binding inhibitory NK cell receptors. Microglial activation by TLR ligation was associated with up-regulation of MHC class I molecules and thereby inhibited NK cell-mediated killing of human MG. These data suggest that brain-infiltrating NK cells might have the capacity, via elimination of resting MG cells, to negatively regulate the afferent limb of innate and adaptive immune responses within the human CNS.

Because of the lack of mouse strains that are selectively deficient in NK cells, the study of NK cell function in vivo has been challenging in the past (35) and animal models of acute inflammatory CNS diseases provided evidence for both disease accelerating and protective effects of NK cells (17, 36–39). It has been suggested that NK cells could be pathogenic by shaping Th1-polarized adaptive immune responses, by activating CNS-infiltrating DCs, and/or via direct recognition and lysis of inflammation-activated brain cells (39, 40). However, most EAE studies reported that NK cells protect from autoimmune-mediated tissue damage, presumably by editing initiator and effector cell populations (15, 16, 37, 41).

Several cell types of the human lymphoid and myeloid lineages have been suggested as targets of autologous NK cell cytotoxicity. T cells have been shown to up-regulate NKG2D ligands following activation and to become susceptible to autologous NK cell-mediated cell lysis in vitro (26, 42, 43). Lu et al. (41) demonstrated that the interaction between the mouse homolog of the human MHC class Ib molecule HLA-E, Qa-1-Qdm, on activated T cells and CD94-NKG2A inhibitory NK cell receptors, protects activated CD4+ T cells from perforin-mediated NK cell cytotoxicity. Ab-dependent blockade of this Qa-1-NKG2A interaction resulted in potent NK-dependent elimination of activated autoreactive T cells and amelioration of EAE in MOG35–55 immunized C57BL/6 mice (41). HLA-E-NKG2A as well as classical MHC class I ligand-killer Ig-like receptor interactions have also been proposed to be involved in the protection of mature DCs from autologous NK cell-mediated killing (44). In addition, activated NK cells can kill autologous immature myeloid DCs via Nkp30-, Nkp46-, and DNAM-1-mediated recognition (31–33, 45–49) and, similar to MG, pathogen infection, TLR ligation, or stimulation by proinflammatory cytokines protect DCs from NK cell-mediated cytotoxicity, presumably via increased surface expression of MHC class I molecules (44–46, 48–50).

In contrast to DCs, monocyte-derived macrophages are resistant to autologous NK cell cytotoxicity unless they become activated and express NKG2D ligands (NKG2DL) by high doses of LPS (200 ng/ml) (19). Consequently, NK cell-mediated cytotoxicity of LPS-stimulated macrophages was abrogated by an anti-NKG2D mAb (19). Although these data indicate that the stimulatory signal resulting from NKG2D-NKG2DL interactions overcomes the inhibitory signal provided by MHC class I ligands on activated macrophages, we observed that microglia stimulated with a high dose of LPS (200 ng/ml) were less susceptible to NK cell-mediated cytotoxicity. Thus, similar to immature and mature DCs, resting MG were susceptible to NK cell-mediated cytotoxicity while microglial activation protected these CNS resident APCs from being killed by NK cells. By this mechanism, NK cells might reduce the microglial pool, but allow fully activated microglial cells to present Ags to infiltrating T cells and to initiate a limited immune response in the brain.

Investigating the interface between CD8+ T cells, NK cells, and oligodendrocytes as a potential mechanisms of immune-mediated tissue injury in multiple sclerosis, Saikali et al. (51) also addressed the susceptibility of fetal brain-derived MG cells toward cell lysis by NK cells. Studying two CNS cell samples, they reported that IL-2-activated NK cells efficiently lyse nonactivated allogeneic MG, but did not find any differences between samples treated with and without NKG2D-specific blocking Abs. In contrast, we found that MG killing by NK cells is substantially and reproducibly inhibited by NKG2D blockade. These differences might be explained by using different clones for Ab-mediated blockade of NKG2D recognition.

Although our findings are compatible with the aggravating effect of NK cell depletion in autoimmune CNS inflammation (15, 37, 41, 52), they do not exclude the possibility that CNS-invading NK cells might display unwanted cytotoxic activity against neuronal cells, oligodendrocytes, or astrocytes. However, experimental data on the interactions between NK cells and CNS resident cells are both scarce and controversial. Oligodendrocytes have been reported to be either susceptible (40, 51) or not (53, 54) toward IL-2-activated NK cell killing. CNS neurons appear to be resistant to NK cell cytotoxicity (55, 56), and Hammarberg et al. (12) reported that CNS-infiltrating NK cells produce high levels of neurotrophic factors, such as brain-derived neurotrophic factor and neurotrophin-3, which protected embryonic motor neuron cultures from TNF-α- or IFN-γ-mediated neuronal cell injury.

In conclusion, we found that NK cells were capable of targeting human MG. These findings extend the functional reach of the NK...
system to include the regulation of CNS intrinsic immune responses. Together with the reported cytotoxic activity of NK cells toward activated T cells, activated macrophages, and immature DCs, our study adds to the evidence for NK cells as immunomodulating lymphocytes involved in the shaping of adaptive immunity beyond their already described polarizing activity via cytokine secretion (57). The mechanism, identified in our experiments might be involved in limiting neuroinflammation associated with BBB disruption and NK cell recruitment into the CNS. Although much has yet to be learned about the function of NK cells during infectious and autoimmune CNS diseases, these data suggest that therapeutic targeting of NK cells or NK cell subsets during CNS inflammation (16) might have clinical merit.

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Disclosures

The authors have no financial conflict of interest.

References


5. Rabinovich, B. A., J. Li, J. Shannon, R. Hurren, J. Chalupny, D. Cosman, and R. G. Miller. 2003. Activated, but not resting, T cells can be recognized and killed by guest on April 13, 2017 http://www.jimmunol.org/ Downloaded from

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