Regulatory NK-Cell Functions in Inflammation and Autoimmunity

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PHENOTYPES AND FUNCTIONS OF NK CELLS IN HUMANS

Although NK cells were described originally as a homogenous population of innate lymphocytes, there is increasing evidence that NK cells include distinct subsets with disparate functions, locations, and developmental origins.

Human NK cells in peripheral blood can be divided into at least two functional subsets based on the expression of CD56 and CD16 (Figure 1). CD56<sup>dim</sup>CD16<sup>+</sup> NK cells constitute about 90% of total blood NK cells, efficiently kill target cells, and secrete only low levels of cytokines. In contrast, CD56<sup>bright</sup>CD16<sup>−</sup> NK cells constitute <10% of total blood NK cells, but are enriched in secondary lymphoid tissues (1). In contrast to the CD56<sup>dim</sup>CD16<sup>+</sup> NK-cell subset, activated CD56<sup>bright</sup>CD16<sup>−</sup> cells produce many cytokines, including IFN-γ, TNF, and GM-CSF, but acquire cytotoxicity only after prolonged activation (2). Therefore, cytokine secretion and cytotoxic effector functions are separated in human NK-cell subpopulations.

CD56<sup>bright</sup>CD16<sup>−</sup> and CD56<sup>dim</sup>CD16<sup>+</sup> NK cells also differ from each other in the expression of inhibitory and activating receptors, as well as in the expression of adhesion molecules and chemokine receptors which facilitate homing to lymphoid tissues and sites of inflammation. NK-cell activation during target cell recognition is controlled by germline encoded activating and inhibitory receptors on these innate lymphocytes. Among these, triggering of killer immunoglobulin-like receptors (KIRs) as well as of the CD94/NKG2A receptor on NK cells by classical and nonclassical MHC class I molecules delivers negative signals that prevent target cell killing (3). In contrast, engagement of activating receptors such as CD16 (FcγRIII), NK cell protein 30 (Nkp30), Nkp44, Nkp46, DNAM-1, and NKG2D by antibody opsonization or ligands whose expression is upregulated following infection, transformation, or cellular stress, provides an NK-cell activating signal (4,5). Of the above-mentioned inhibitory receptors, cytokine-secreting CD56<sup>bright</sup>CD16<sup>−</sup> NK cells express high levels of the inhibitory CD94/NKG2A complex recognizing HLA-E, but lack MHC class Ia allele-specific killer cell Ig-like receptors, which are, in contrast, expressed by CD56<sup>dim</sup>CD16<sup>+</sup> NK cells. Both NK-cell subsets express the activating receptors NKG2D as well as Nkp30 and Nkp46, but expression of the antibody-dependent cellular cytotoxicity mediating receptor CD16 (FcγRIII) is confined to the CD56<sup>dim</sup>CD16<sup>+</sup> subset (6). Therefore, cytokine secreting CD56<sup>bright</sup>CD16<sup>−</sup> NK cells express a more rudimentary repertoire of inhibitory NK-cell receptors than cytotoxic CD56<sup>dim</sup>CD16<sup>+</sup> NK cells. CD56<sup>bright</sup>CD16<sup>−</sup> NK cells constitutively express CD62L (L-selectin) and...
Human NK cells in peripheral blood can be divided into at least two subsets based on the expression of CD56 and CD16. The major subset of CD56<sup>bright</sup>CD16<sup>+</sup> NK cells constitutes ~90% of total blood NK cells, kills target cells upon proper recognition, and secretes only low levels of cytokines. CD56<sup>bright</sup>CD16<sup>+</sup> NK cells (~10% of total blood NK cells, but 75% of NK cells in secondary lymphoid tissues), in contrast, produce many cytokines, including IFN-γ, TNFα, and GM-CSF upon stimulation by proinflammatory cytokines, but acquire cytotoxicity only after prolonged activation. In addition, both subsets differ from each other with respect to expression of inhibitory and activating receptors. Although CD56<sup>bright</sup>CD16<sup>+</sup> NK cells express high levels of the inhibitory CD94/NKG2A complex recognizing HLA-E, they do not express MHC class I allele-specific killer cell Ig-like receptors that are, in contrast, expressed by CD56<sup>dim</sup>CD16<sup>+</sup> NK cells. Moreover, only CD56<sup>bright</sup>CD16<sup>+</sup> NK cells express homing markers for secondary lymphoid organ (SLO), such as CCR7 and CD62L and immature lymphocyte markers, such as CD94/NKG2A complex recognizing HLA-E (32), suggesting that cell contact might mediate priming and expansion of CD8<sup>+</sup> T cells (28). On the other hand, activated NK cells can kill autologous immature myeloid DCs via NKp30-, NKp46-, and DNAM-1-mediated recognition (29–31). Resistance to NK lysis was achieved via upregulation of MHC class I molecules upon DC maturation, in particular, upregulation of HLA-E (32), suggesting that NK cells expressing the inhibitory recep-
 tol for HLA-E recognition, that is, CD94/NKG2A, are particularly important in editing nonimmunogenic DCs. In addition to DCs, other myeloid cells also have been found to be susceptible to NK-cell cytotoxicity. Along these lines, activated macrophages have been found to be susceptible to NKG2D-dependent cytotoxicity by NK cells (33), and we have shown recently that human microglial cells, resident macrophage-like antigen presenting cells (APCs) of the central nervous system (CNS), are lysed by activated NK cells via NKG2D- and Nkp46-mediated recognition (34). Similar to immature DCs, resting microglia are susceptible to NK-cell-mediated cytotoxicity while microglial activation and up-regulation of MHC class I molecules protected these CNS-resident APCs from being killed by NK cells (34). We suggest that this mechanism reduces the pool of immature DCs and resting microglial cells during immune activation, but allows fully activated APCs to present antigens to infiltrating T cells and to initiate a limited immune response. In contrast to DCs and microglia, monocye-derived macrophages are resistant to autologous NK-cell cytotoxicity, unless they become activated by high doses of lipopolysaccharide (LPS), and express NKG2D ligands (NKG2DL) (33). This indicates that the stimulatory signal resulting from NKG2D–NKG2DL interactions overcomes the inhibitory signal provided by MHC class I ligands during recognition of activated macrophages by NK cells. The latter study also indicates fundamental differences between different subsets of myeloid cells in their susceptibility toward NK-cell–mediated killing. Although all of the above mentioned studies were performed in vitro, and definite evidence that these mechanisms are also operative in vivo is still lacking, the results obtained suggest that activated NK cells might play an important regulatory role by selectively editing APCs during the course of immune responses.

In addition to regulation of myeloid cells by NK cells, several studies also have shown the importance of NK cells for promoting Th1 polarization of CD4+ T cells through production of IFN-γ, a cytokine that not only initiates, but also reinforces Th1 differentiation. During experimental Leishmania major infection, NK cells are recruited rapidly to lymph nodes where they are found in close contact to the same DCs as antigen-specific CD4+ T cells providing the IFN-γ required for the induction of Th1 polarization (17). A similar effect was observed in allogeneic immune responses during which NK cells produce high levels of IFN-γ that are sufficient to mediate T-cell polarization via acting on naïve T cells directly, and by enhancing DC maturation (35–37). In addition to their function in T-cell polarization, NK cells expressing OX40 ligand and CD86 upon ligation of the activating FcγRIII (CD16) are capable of inducing IFN-γ production and proliferation of autologous T cells (38). Moreover, a supportive role of NK cells in B-cell activation and the promotion of isotype class switching has also been documented (39-41). These studies indicate that NK cells, particularly the cytokine secreting CD56brightCD16- subset (37), can influence T-cell polarization during primary immune responses.

In addition to shaping T-cell responses, NK cells can, however, also dampen these. Following T-cell activation, T cells upregulate NKG2D ligands and become susceptible to autologous NK-cell–mediated cell lysis in vitro (42–44). In this context, Lu et al. (45) demonstrated that the interaction between the mouse homologue 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 experimental autoimmune encephalomyelitis (EAE) in myelin oligodendrocyte glycoprotein (MOG) 35–55 immunized C57BL/6 mice (45). Thus, NK cells contribute to the resolution of adaptive immune responses via deletion of activated T cells in vivo. The latter study provided a mechanistic insight into the finding that NK-cell depletion profoundly influences the outcome of T-cell–mediated autoimmune disease models as outlined below.

**NK-CELL FUNCTION IN EXPERIMENTAL AUTOIMMUNE DISEASES**

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 (46). Animal models of autoimmune diseases provide evidence for both disease-accelerating and disease-protective effects of NK cells (Figure 2). The contradictory results achieved by NK-cell depletion are best illustrated in EAE, the animal model of multiple sclerosis (MS). It has been suggested that NK cells could be pathogenic by shaping Th1-polarized adaptive immune responses and by activating CNS-infiltrating DCs (47). Indeed, several mouse strains which have defective NK-cell functions, such as IL-18 deficient mice, or that are devoid of NK cells, such as T-bet deficient mice, are resistant to EAE induction (48,49). Surprisingly, most studies in the EAE model reported that NK cells protect from autoimmune-mediated tissue damage, presumably by editing initiator and effector cell populations (45,50–52). Antibody-mediated depletion of NK cells exacerbated EAE pathology in Lewis rats, SJL mice, and C57BL/6 mice (50,51,53), whereas adoptive transfer of in vitro-generated NK cells from the bone marrow decreased the severity of EAE in DA rats (54). Furthermore, it was observed that NK-cell depletion resulted in increased proliferation and IFN-γ production of T cells specific for the antigen that was used to induce EAE (50). Interestingly, opposite findings were observed in a different study using the same mouse strain immunized with the same myelin antigen (47). Contradictory findings on the effects of NK-cell depletion also were reported in animal models of type 1 diabetes (T1D). Whereas antibody-mediated NK-cell depletion was protective in one study (55), another report suggested that
mice which allow selective in vivo initiation and progression. Studies in that mediate divergent effects on EAE potentially target distinct subsets of NK cells and IFN-γ antibody-mediated NK-cell depletion cytotoxic T cells, respectively. Moreover, killer T cells (NKT cells) and a subset of which also are expressed on natural depletion target NK1.1 and asialo-GM1, frequently used antibodies for NK-cell targets NK cells specifically. The most outcomes. Unfortunately, no antibody NK-cell depletion could explain different different tools and protocols to achieve established disease. In addition, using sJRA showed decreased NK-cell frequencies and impaired NK-cell functions compared with children with other forms of juvenile rheumatoid arthritis such as pauciarticular and polyarticular JRA, even in the absence of MAS (71,72), which can, however, be triggered by environmental insults such as viral infections (73). Thus, patients with HLH and sJRA represent an example for an association of NK-cell dysfunction and excessive immune activation, which could contribute, potentially, to the initiation and maintenance of autoimmune responses.

On the other hand, there are many studies which found no differences in NK-cell frequencies and functions in patients with common autoimmune diseases compared with healthy controls (74–77). These contradictory findings are difficult to interpret since all of the aforementioned studies differed widely in their criteria used to classify NK cells. Some of the older reports did not distinguish between NK cells and NKT cells and none of the above studies differentiated between CD56brightCD16− and CD56dimCD16+ NK cells. Moreover, the assays and protocols used to study NK cell frequencies and functions, as well as the patient populations studied, varied significantly (78). With these caveats in mind, genetic predisposition for autoimmune diseases has been linked to NK cells, and successful therapies of autoimmune diseases correlated with changes in the NK-cell compartment.

The genetic analysis of NK cells has focused mainly on KIR allele typing, KIRs are expressed on NK cells and subsets of T cells. The KIR genes are extremely polymorphic, probably more than the HLA loci, and the KIR gene complex is polygenic with varying numbers of inhibitory and activating receptors (79). HLA class I molecules serve as ligands for the KIR. Interactions of the independently segregating KIR and HLA loci are important for recognition of targets by NK cells. Several studies correlated incidence and progression of infectious and autoimmune diseases with the expression of particu-
lar KIRs, as well as with HLA–KIR combinations. For example, in patients with psoriasis and psoriatic arthritis, conditions that have long been associated with HLA-C, specifically with the C2 allotype gene HLA-C*06, it has been reported that activating KIR genes (usually KIR2DS1 and/or KIR2DS2) are at a higher frequency in patients than in healthy control individuals (80,81). These studies suggest that the overall balance of activating and inhibitory composite KIR–HLA genotypes contributes to the susceptibility toward autoimmune diseases. However, it remains to be defined whether these genotypes also translate into functional differences (82).

Stronger evidence for a regulatory role of NK cells in MS stems from immunomonitoring of a phase II therapy trial in MS. The rationale for the use of a humanized IgG1 monoclonal antibody targeting the interleukin-2 receptor \( \alpha \) chain (daclizumab) in patients with autoimmune diseases such as MS was to block the proliferation of antigen-activated autoreactive T cells. Indeed, results of four open-label studies of intravenous daclizumab in patients with active forms of either RRMS or secondary progressive MS (SPMS) have suggested beneficial effects of daclizumab for both add-on and monotherapy protocols as measured by magnetic resonance imaging (MRI) and clinical outcomes (83). Surprisingly, CD4+ and CD8+ T-cell counts were decreased only moderately in patients responding to therapy. In stark contrast, CD56brightCD16+ NK cells increased in frequency in the blood of treated patients and the suppression of disease activity, as quantified by MRI, correlated with the expansion of blood NK cells (84). Notably, NK cells isolated from patients during, but not before, therapy killed autologous-activated T cells even without the need for NK-cell activation in vitro (84). Similar observations were made in patients who received the identical monoclonal antibody for the treatment of autoimmune uveitis (85). In addition, patients with MS receiving IFN-\( \beta \) therapy also show an increase in the frequency of CD56brightCD16+ NK cells within 3 months of treatment (86). Altogether, these human studies suggest that NK cells exert beneficial functions in autoimmune diseases. The mechanisms that could mediate such immunoregulatory NK-cell functions remain, however, poorly understood.

Nevertheless, both genetic and immunotherapeutic observations point to an involvement of NK cells in autoimmune disease and therefore their role in these pathologies needs to be better understood.

**CONCLUSION**

NK cells are multicompetent lymphocytes with the ability to regulate innate and adaptive immune responses through their reciprocal interaction with antigen-presenting cells as well as T cells and B cells. Antibody-mediated NK-cell depletion profoundly affects the outcome of many experimental autoimmune diseases. The observation that NK cells are decreased in frequency or impaired in function in patients with autoimmune diseases are reminiscent of defective functions noted for regulatory T-cell populations such as CD4+CD25+ regulatory T (Treg) cells and CD1d-restricted NKT cells, in patients with autoimmune diseases (87–90).

NK cells include distinct subsets with disparate repertoires, location, function, and developmental origin. In analogy to regulatory T-cell subsets, NK-cell subpopulations that have yet to be characterized might be particularly important in regulating autoimmune inflammation. We suggest that investigating immunoregulatory NK-cell functions in healthy individuals and patients with autoimmune diseases will generate exciting insights into the reciprocal regulation between NK-cell–mediated innate immunity and adaptive immune responses, improve our capacity to monitor these cells as surrogate markers for disease activity and treatment response, and, perhaps, provide new prospects for NK-cell–directed therapies.

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**DISCLOSURE**

The authors declare that they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

**REFERENCES**


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