Published in final edited form as:

Expert Rev Anti Infect Ther. 2008 December ; 6(6): 867–885. doi:10.1586/14787210.6.6.867.

Natural killer cells in immunodefense against infective agents

Nicolas Zucchini,

Centre d'Immunologie de Marseille-Luminy, Université de la Méditerranée, Marseille, France and Institut National de la Santé et de la Recherche Médicale (INSERM), U631, Marseille, France and Centre National de la Recherche Scientifique (CNRS), UMR6102, Marseille, France, Tel.: +33 491 269 461, Fax: +33 491 269 430, zucchini@ciml.univ-mrs.fr

Karine Crozat, PhD,

Centre d'Immunologie de Marseille-Luminy, Université de la Méditerranée, Marseille, France and Institut National de la Santé et de la Recherche Médicale (INSERM), U631, Marseille, France and Centre National de la Recherche Scientifique (CNRS), UMR6102, Marseille, France, Tel.: +33 491 269 461, Fax: +33 491 269 430, crozat@ciml.univ-mrs.fr

Thomas Baranek, PhD,

Centre d'Immunologie de Marseille-Luminy, Université de la Méditerranée, Marseille, France and Institut National de la Santé et de la Recherche Médicale (INSERM), U631, Marseille, France and Centre National de la Recherche Scientifique (CNRS), UMR6102, Marseille, France, Tel.: +33 491 269 461, Fax: +33 491 269 430, baranek@ciml.univ-mrs.fr

Scott H Robbins, PhD,

Centre d'Immunologie de Marseille-Luminy, Université de la Méditerranée, Marseille, France and Institut National de la Santé et de la Recherche Médicale (INSERM), U631, Marseille, France and Centre National de la Recherche Scientifique (CNRS), UMR6102, Marseille, France

Marcus Altfeld, MD, PhD, and

Partners AIDS Research Center and Infectious Disease Unit, Massachusetts General Hospital and Division of AIDS, Harvard Medical School, Boston, MA, USA, Tel.: +1 617 724 2461, Fax: +1 617 724 8586, maltfeld@partners.org

Marc Dalod, PhD[†]

Centre d'Immunologie de Marseille-Luminy, Université de la Méditerranée, Marseille, France and Institut National de la Santé et de la Recherche Médicale (INSERM), U631, Marseille, France and Centre National de la Recherche Scientifique (CNRS), UMR6102, Marseille, France, Tel.: +33 491 269 466, Fax: +33 491 269 430, dalod@ciml.univ-mrs.fr

Abstract

Following the discovery of innate immune receptors, the topics of innate immunity and its role in defense against infective agents have recently blossomed into very active research fields, after several decades of neglect. Among innate immune cells, natural killer (NK) cells are endowed with the unique ability to recognize and kill cells infected with a variety of pathogens, irrespective of prior sensitization to these microbes. NK cells have a number of other functions, including cytokine

[†]Author for correspondence: Centre d'Immunologie de Marseille-Luminy, Parc Scientifique de Luminy, Case 906, F-13288 Marseille Cedex 09, France, Tel.: +33 491 269 466, Fax: +33 491 269 430, E-mail: dalod@ciml.univ-mrs.fr.
Current address: Genomics Institute of the Novartis, Research Foundation, John Jay Hopkins Drive, San Diego, CA 92121, USA, Tel.: +1 858 812 1690, Fax: +1 858 332 4502, srobbins@gnf.org

Financial & competing interests disclosure: The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

production and immunoregulatory activities. Major advances have recently been made in the understanding of the role of NK cells in the physiopathology of infectious diseases. The cellular and molecular mechanisms regulating the acquisition of effector functions by NK cells and their triggering upon pathogenic encounters are being unraveled. The possibility that the power of NK cells could be harnessed for the design of innovative treatments against infections is a major incentive for biologists to further explore NK cell subset complexity and to identify the ligands that activate NK cell receptors.

Keywords

hemophagocytic lymphohistiocytosis; IL-12; IL-15; intracellular bacteria; killer cell immunoglobulin-like receptor; natural cytotoxicity receptor; natural killer cell; protozoan parasite; type I interferon; virus

In mammals, the defense against infective agents involves two distinct arms of the immune system: the innate and adaptive responses. Innate immunity can be activated very rapidly upon pathogen entry to mount effector and immunoregulatory responses, without the need for prior sensitization. It is based on germline-encoded receptors able to detect different types of danger signals, either from microbial origin (referred to as 'nonself' or 'pathogen-associated molecular patterns' [PAMPs]) or from host origin and induced by the infection (referred to as 'modified self' or 'danger-associated molecular patterns' [DAMPs]) [1]. Receptors of the innate immune system exhibit a very broad specificity and are often highly conserved throughout evolution. By contrast, receptors involved in the adaptive immune response have emerged more recently from an evolutionary perspective and are generated through gene recombination of complex loci, which leads to an extreme diversity of receptors, each characterized by a focused antigen specificity. Adaptive immunity is subordinate to innate immunity because conventional B and T lymphocytes require activation by innate immune cells, in particular interactions with dendritic cells (DCs) and a proper cytokine milieu, in order to be able to respond adequately to the first encounter with a given antigen. Thus, innate immunity is critical for both an immediate confinement of the infectious agent and the induction of efficient primary and memory adaptive immune responses. The cells of the adaptive immune system belong to two major leukocyte subsets, both of lymphoid origin, B cells and T cells. By contrast, the innate immune system is composed of many leukocyte subsets that differ broadly in their ontogeny, activation requirements and functions. Cells of the innate immune system include DCs, cells of myeloid origin, such as monocytes/macrophages and neutrophils, as well as cells from lymphoid origin, including natural killer (NK) cells. This review focuses on the role of NK cells in immunodefense against infections.

Natural killer cells are cytotoxic lymphocytes that are able to kill target cells expressing PAMPs or DAMPs as a consequence of infection or cellular transformation. In particular, NK cells have long been known to be able to kill virus-infected cells *in vitro* [2] and to contribute to the control of the replication of different viruses *in vivo* in mice [3] and humans [4]. In addition to their cytolytic capabilities, NK cells are also able to secrete a number of cytokines, including IFN- γ , as well as multiple chemokines. One objective of NK cell biology research has been the acquisition of sufficient knowledge to allow the power of NK cells to be harnessed for vaccination and therapeutic interventions in the clinic. Recently, many significant advances have been made in our understanding of the molecular mechanisms that regulate NK cell functions and in the investigation of their role in the physiopathology of infections by different types of intracellular pathogens. This review aims to describe these advances and provide a framework in which this knowledge could potentially be exploited to develop innovative vaccines or immunotherapeutic strategies. As NK cell control of viral and parasitic infections have a number of commonalities, we have discussed these two types of infections together.

For a more detailed analysis of the specificities of NK cell responses to different classes of pathogens, we refer the reader to the recent review by Lodoen and Lanier [5].

First, we briefly summarize the general knowledge regarding the basics of NK cell biology, mainly how these cells can be identified, what their functions are and how they are regulated. We proceed to a focused recapitulation of the evidence that NK cells are activated, and take part in the control of pathogen replication, during a variety of infections. We then summarize what is known of the conditions regulating NK cell functions in selected infections, and discuss the protective versus detrimental roles of NK cell functions in these settings. Finally, we discuss how NK cell functions could be modulated therapeutically to help promote health over disease during infections. This review is not meant to be an exhaustive source on the subject of NK cells in immunodefense against infective agents. Rather, through illustration of exemplary infectious models, it aims to emphasize key concepts, either firmly established or currently emerging, and to propose tentative directions for future investigations.

Generalities on the biology of NK cells

Natural killer cells constitute a specific lymphocyte subset that differentiates early on from a hematopoietic progenitor common to T lymphocytes [6]. Until recently, the identification of NK cells has relied on their lack of expression of CD3, combined with their expression of CD16 or CD56 in humans, of NK1.1 in C57BL/6 mice or of CD49b (DX5) in all mouse strains. However, these combinations of markers may not always identify NK cells unambiguously. Other cell types can harbor an overlapping phenotype, especially in the context of infections. Recently, the Nkp46 molecule has been demonstrated to be the most specific and universal NK cell marker in mammals [7]. Its use moving forward should help to improve consistency within the research community with regard to identifying and studying NK cells in multiple model systems. Several NK cell subsets can be defined according to their tissue distribution, state of maturation or functional specialization. For example, in humans, two NK cell subsets have been reported in the blood: the CD3⁻CD56^{dim}CD16⁺ subset is endowed with a strong cytotoxic activity, whereas the CD3⁻CD56^{bright}CD16⁻ subset is more prone to cytokine production (as reviewed in [8]), and is present in significant numbers in the lymph nodes [9].

Natural killer-cell effector functions are mainly regulated by three classes of signals: cytokines, activation receptors and inhibitory receptors. NK cells have also been demonstrated to express a number of functional Toll-like receptors (TLRs) [10]. Cytokines appear to be critical for the rapid and widespread acquisition of the machinery required for NK cell effector functions: the synthesis of cytotoxic granules and of cytokines, as well as for NK cell survival and proliferation. The balance between the signals originating from inhibitory versus activation receptors regulates NK cell effector functions upon encounter of potential target cells, depending on the pattern of ligands these cells express (reviewed in [11]). Two families of functionally homologous but nonorthologous NK cell receptors have been reported in mammals. They are characterized by the presence of paired activating versus inhibitory molecules presenting a high sequence homology of their extracellular domain for ligand recognition but differing in their transmembrane and intracellular signaling motifs (reviewed in [11]). Humans express the killer cell immunoglobulin-like receptors (KIRs), while mice express the lectin-like Ly49 dimers. In both species, inhibitory receptors also include the lectin-like CD94–NKG2A heterodimers, and activation receptors include the natural cytotoxicity receptor (NCR) Nkp46. Human NK cells also express the activating lectin-like CD94–NKG2C receptor and the NCRs Nkp30 and Nkp44. Most NK cell inhibitory receptors recognize host MHC class I molecules. Under homeostatic conditions, this prevents NK cells from destroying healthy cells that express normal levels of these molecules. During cellular transformation or viral infection, the downmodulation of MHC class I expression often occurs. This downmodulation favors NK cell activation by removal of this constitutive break mediated by

inhibitory receptors. Other inhibitory receptors have recently been demonstrated to recognize non-MHC self molecules [11].

The physiological relevant specificities of most of the activating NK cell receptors are still unknown. A noticeable exception is NKG2D, which recognizes a family of DAMP molecules inducible in a variety of cell types [12]. For example, NKG2D ligands can be induced by viruses, either as a direct consequence of their replication in infected cells or indirectly in response to type I interferons (IFNs) [13], which are the most prominent and characteristic cytokines induced by viral infections.

Evidence of the modulation of NK cell functions by infections

Natural killer cells have long been demonstrated to be activated *in vitro* by virus-infected cells [2,14]. Other types of intracellular pathogens have also been shown to activate NK cells for IFN- γ production or enhance cytotoxicity against tumor cell lines, as reviewed recently [5, 15]. Evidence for an eventual implication of NK cells in the control of extracellular pathogens is scarce [16].

Chronic viral infections have been shown to alter the relative proportions of NK cell subsets or their functions [17–19]. In acute HIV-1 infection, the CD56^{bright} NK cell subset is replaced by CD56^{dim} NK cells with decreased effector functions. This early deregulation of NK cells is followed in the chronic phase of the infection by a progressive increase in functionally anergic CD56⁺CD16⁺ NK cells. This functional anergy is due, at least in part, to an increased expression of the inhibitory transduction molecule SHIP-1 and to a decreased expression of perforin [20]. It is not yet clear whether this phenomenon contributes to the failure of the host to control HIV-1 or whether it is merely a consequence of a chronic imbalance in the cytokine milieu due to immunomodulatory functions of viral proteins within infected cells [21], or to the overwhelming activation of proinflammatory cells induced by the chronic replication of the virus [22].

Thus, as they are activated by a variety of intracellular pathogens, including many viruses and also bacteria or protozoa, NK cells have the potential to contribute to the immune defense against a variety of infections. However, in certain infections that lead to a high induction of NK cell activation, there is no evidence that NK cells play a direct role in the control of the pathogen (e.g., during lymphocytic choriomeningitis virus [LCMV] infection [3]). Thus, the modulation of NK cell functions by a given infection is not sufficient to indicate that NK cells contribute directly to the control of the corresponding pathogen. Direct evidence for the actual implication of NK cells in the control of infections are discussed in the next section.

Evidence of NK cell implication in the control of infections

Impact of congenital NK cell deficiencies or of artificial depletion of NK cells on the control of infections

To the best of our knowledge, the first direct evidence of the implication of NK cells in the control of viral infections *in vivo* came from studies of murine cytomegalovirus (MCMV) infection. These studies showed that a defect in cellular degranulation, due to a spontaneous mutation in the *Lyst* gene in the beige mouse strain, resulted in an increased susceptibility to MCMV infection. Indeed, compared with control littermates, beige mice succumbed very early after viral challenge, before the onset of adaptive immunity [23]. Shortly afterwards, the *in vivo* treatment of mice with the anti-AGM1 antibody was demonstrated to deplete NK cells in a relatively specific manner and to cause a significant increase in viral replication in the liver upon challenge with MCMV, mouse hepatitis virus (MHV) or vaccinia, but not LCMV [3]. Simultaneously, NK cells were also shown to play an important role in the control of the

morbidity and the mortality caused by influenza infection [24]. The use of depletion protocols with a better, although not absolute, specificity for NK cells, mainly anti-NK1.1 antibodies in C57BL/6 mice, have since firmly confirmed the implication of NK cells in the control of the replication of a number of viruses, including MCMV [25,26], herpes simplex virus (HSV)-1 [27], HSV-2 [28] and Theiler's virus [29]. The selective *in vivo* depletion of NK cells has also demonstrated their implication in immunodefense against other types of infectious agents, including the control of the parasite *Plasmodium berghei* (reviewed in [30]) and resistance to *Toxoplasma gondii*-induced mortality [31]. The recent generation of a transgenic mouse model expressing the diphtheria toxin receptor under the control of the regulatory regions of human NKp46 should allow an easier and more precise investigation of NK cell implication in the resistance to a variety of infections on different genetic backgrounds [7]. Since the seminal publication from Biron and colleagues [4], investigations into the basis for enhanced sensitivity to viral infections in humans has led to the identification of selective NK cell deficiencies in a number of individuals, and revealed congenital mutations in molecules critical for NK cell development or functions [32,33]. Interestingly, HIV-1-exposed uninfected individuals have been suggested to have enhanced defenses to the virus due, at least in part, to stronger NK cell activity [34–36].

Correlations between haplotypes for the NK cell receptor & MHC loci & resistance to infection

Other evidence for the implication of NK cells in the control of infections has come from correlative studies designed to examine the relationship between disease severity and genetic haplotypes for candidate loci. To the best of our knowledge, the first association between the haplotype of a NK cell locus and the ability to resist a viral infection was reported in 1995 by Scalzo and colleagues, who mapped the resistance of the C57BL/6J mouse strain to MCMV infection to the NK cell receptor locus (Cmv1r) on chromosome 6 [25]. The development of the BXD-8 congenic mouse strain was determinant for the identification of the activation receptor involved in C57BL/6 NK cell recognition of MCMV-infected cells [26,37]. Scalzo and colleagues developed other congenic mouse strains bearing parts of the C57BL/6 NK cell locus in a BALB/c genetic background [38], which have been extremely useful to correlate NK cell locus-associated polymorphisms to resistance to various pathogens including HSV-1 [39] and *P. berghei* [40]. However, while the Cmv1r allele is directly associated with the control of MCMV load [41], it is not involved directly in the control of *P. berghei* replication but prevents the development of different symptoms induced by the infection with this parasite, such as cerebral malaria, pulmonary edema and severe anemia [40].

In humans, a number of correlation studies examining disease severity and haplotypes for the NK cell and MHC loci have been performed recently, particularly by Carrington's group (reviewed in [42]). These analyses suggest the implication of specific NK cell receptors and their ligands in the control of disease progression during viral infections, including HIV-1 [43], human hepatitis C virus (HCV) [44], HSV-1 [45] or influenza [46] infections, as well as in the resistance to malaria [30]. The hypothesis that an epistatic interaction between the activation receptor KIR3DS1 and its presumed ligand HLA-B Bw4–80I plays a protective role in the control of the progression towards AIDS in HIV-1-infected patients has since been supported by the observation that KIR3DS1⁺ NK cells control viral replication more efficiently in CD4 T cells expressing HLA-B Bw4–80I, in an *in vitro* setting [47]. Of note, the expression of KIR3DS1 has recently been demonstrated to be associated with enhanced NK cell function and decreased CD8 T-cell activation during early HIV-1 infection, irrespective of the expression of HLA-B Bw4–80I, which may lead to a better clinical outcome [48]. Finally, an increased proportion of KIR3DS1 homozygotes has been reported very recently in HIV-exposed uninfected individuals [36]. However, the identification of the ligand for KIR3DS1 is still pending, and the molecular mechanisms underlying the KIR3DS1-mediated recognition of HIV-1-infected cells by NK cells are not known.

Evolutionary selection of mechanisms of pathogen escape to NK cell responses

Another evidence of the general importance of NK cells in the control of viral infections comes from the observation that viral genomes encode molecules that antagonize NK cell functions (reviewed in [49]). Inactivation of these viral molecules confers NK cell-dependent enhanced resistance of the host. The strategies evolved by viruses to escape host NK cell responses are diverse. They include the downregulation of NKG2D ligands at the surface of infected cells, production of soluble NKG2D ligand antagonists, expression of molecules mimicking MHC class I or other host ligands for NK cell inhibitory receptors, selective downregulation of HLA-A and HLA-B by HIV-1 negative factor (nef), but maintained expression of HLA-C or the expression of IL-18 antagonists. This demonstrates that host/pathogen coevolution has led to the selection of viral variants that have acquired the ability to escape NK cell responses. Thus, this implies that NK cell responses are a major driving force in the coevolution between viruses and their mammalian hosts, likewise to type I IFN responses [50].

In conclusion to this section, the involvement of NK cells in the *in vivo* control of viral replication is supported by a large body of evidence obtained from studies performed in both mouse and human systems. Additional studies are also accumulating using *in vitro* or *in vivo* mouse models that demonstrate a role for NK cells in immunodefense against other intracellular pathogens, mostly protozoa but also intracellular bacteria. Currently, the significance of NK cells in the natural history of human infections with these pathogens remains unclear. The next two sections will summarize current knowledge on the conditions that promote the acquisition and the triggering of NK cell effector functions during infections, which is summarized in Figure 1A.

Conditions of NK cell activation & recruitment during infections

Natural killer cells were initially described as able to exert potent cytotoxic activity against target cells without the need of prior activation, in contrast to cytotoxic CD8 T lymphocytes, which require priming by DCs. However, during viral infections, type I IFN responses are strictly required to promote efficient NK cell antiviral activity. During MCMV infection, nonredundant functions of different innate cytokines directly control distinct NK cell functions: IL-12 is required for IFN- γ production, type I IFN for enhanced cytotoxicity and IL-15 for survival and proliferation [51]. Type I IFNs are produced mainly by plasmacytoid DCs at the time of initiation of NK cell activation, approximately 1.5 days after infection, while IL-12 can be produced by all DC subsets and by monocytes/macrophages, depending on the mouse strain [52]. Infected cells from the splenic stroma can also bear a significant contribution to the production of type I IFN in immunocompetent animals in the first 12 h after infection, significantly prior to any detectable activation of NK cells [53]. Interestingly, NK cell activation by type I IFN can occur either by direct action of the cytokines on the NK cells themselves, as in the case of vaccinia infection [54], or in part indirectly through the activation of DCs or monocyte/macrophages for IL-15 production and cross-presentation to NK cells [55–57]. IL-18 can synergize with IL-12, IL-15 or type I IFN to amplify IFN- γ production [58], proliferation [59] or cytotoxicity [60] by NK cells during viral infections.

Natural killer-cell activation in response to infections by intracellular bacteria, such as *Listeria monocytogenes* [55], or protozoa, such as *Leishmania* [61] or *Plasmodium* [58,62], appears to similarly involve the production of IL-12 and IL-18 by DCs or monocyte/macrophages as well as direct interactions between these ‘accessory cells’ and NK cells. Crosslinking of activation receptors on primary NK cells can contribute to the induction of IFN- γ production and even to cytotoxic activity [63,64]. However, deficiencies in type I IFN, IL-12, IL-15 or IL-18 responses lead to severe defects in NK cell functions in infected animals (reviewed in [14]). Thus, (as reviewed in [65,66]), under physiological conditions, innate cytokines and accessory cells appear critical for the rapid and widespread acquisition of the machinery required for NK

cell effector functions: the induction of IFN- γ production and the translation of pre-existing pools of granzyme B and perforin mRNA [67], as the stimulation through activation receptors is insufficient for this function. As discussed later, the major role of the activating NK cell receptors may be more that of a downstream recognition system to trigger NK cell effector functions at the right time and the right place. Finally, NK cells can respond to a variety of chemokines that are essential for their recruitment to the inflammation sites soon after infection, as demonstrated in the model of MCMV infection in a seminal report from Salazar-Mather and colleagues [68], more recently in *T. gondii* infection [31] and reviewed elsewhere [69].

Conditions of pathogen recognition by NK cells

Altered self recognition

The best characterized mechanisms implicated in NK cell recognition of target cells is the detection of ‘missing self’, as initially proposed by Kärre in 1981 [70]. Most of the NK cell inhibitory receptors are specific for host MHC class I molecules, such that NK cell effector functions are dynamically inhibited by normal cells through this receptor–ligand interaction. A decrease in MHC class I expression that can occur on transformed or virally infected cells alleviates this inhibition and allows triggering of NK cell effector functions. Therefore, NK cells detect infected cells, in part, by monitoring MHC class I expression on their surface. Other self molecules can play a similar role in specifying, through their normal expression, the integrity of the cell. For example, in the rat, the lectin ligand of the NK cell activation receptor NKR-P1B is downmodulated during cytomegalovirus (CMV) infection and replaced by a viral decoy that maintains NK cell inhibition through engagement of NKR-P1B. The genetic inactivation of this viral ligand is sufficient to allow the control of the infection by NK cells [71]. However, in most instances, a reduced expression of MHC class I is not sufficient *per se* to allow efficient killing of target cells by NK cells. The engagement of an activation receptor is also required, as demonstrated by the failure of NK cells deficient in activation receptors, or in the signaling pathways downstream of these activation receptors, to kill a number of target cells expressing low levels of MHC class I [63,72,73]. Moreover, in certain contexts, signals emanating from activation receptors can overcome signals from inhibitory receptors [11].

The best characterized specificity of NK cell activation receptors is that of NKG2D, which monitors the expression of self molecules induced by a variety of stress signals on a broad range of host cell types. NKG2D ligands encompass the UL16 binding protein 1 (ULBP), MICA/B and RAET1G molecules in humans, and the Rae, H60 and MULT1 molecules in mice [12]. These molecules can be induced by innate cytokines, including type I IFN [13], or by DNA damage [74]. As their name reveals, the ULBP proteins have been initially identified as ligands for the UL16 protein of human CMV (reviewed in [49]). Indeed, UL16 affects NK cell recognition of CMV-infected cells by preventing cell surface expression of NKG2D ligands. Likewise, MCMV encodes for several proteins that prevent the cell surface expression of mouse NKG2D ligands and prevent efficient recognition of infected targets by NK cells in sensitive mouse strains lacking other activation receptors for NK cell detection of MCMV-infected targets, such as BALB/c animals. MCMV viruses deleted of these genes become susceptible to NK cell-mediated control [49]. This implies that NKG2D-dependent NK cell recognition of virally infected cells is a major driving force in the coevolution between viruses and their mammalian hosts. It has been proposed that some NK cell activation receptors could recognize a complex between self MHC class I molecules and a viral peptide. Indirect evidence in support of this hypothesis include the epistatic interactions between alleles of a NK cell activation receptor and a MHC class I molecule, such as described for HIV-1 infection in humans [42,47] or for MCMV infection in the mouse [75], as well as biochemical data demonstrating peptide dependent variations in the binding affinity of activating [76] and inhibitory [77,78] KIRs to HLA molecules.

Infectious nonself-recognition

As mentioned earlier, the physiologically relevant specificities of most of the activating NK cell receptors are still unknown. It has been proposed that some NK cell activation receptors bind directly to molecules encoded by infectious agents. The strongest argument in favor of this concept is the recent discovery that Ly49H, the activating NK cell receptor conferring enhanced resistance to MCMV infection in C57BL/6 mice, is directly engaged and triggered by m157, a viral molecule expressed at the surface of infected cells and harboring an MHC class I-like tertiary structure [79,80]. Indeed, m157 deletion abrogates the NK cell-dependent resistance to MCMV infection of C57BL/6 mice [81], even though a high systemic activation of NK cells is observed in terms of IFN- γ production and cytotoxic granule expression. Thus, this demonstrates that m157 is the only MCMV-encoded activating ligand for Ly49H. It also demonstrates that Ly49H is not required for NK cells to acquire the machinery necessary for the synthesis of cytokines and of cytotoxic granules but that it is critical to allow specific recognition of infected cells and consecutive triggering of effector functions. This mechanism of direct recognition of a pathogen-encoded molecule could represent an extraordinary exception to the general rule that NK cell Ly49 or KIR activation receptors may be molecules specialized for the detection of altered MHC class I, as discussed in the previous section (reviewed in [82]).

Human NKp46 and NKp44 have been reported to bind the hemagglutinin of influenza or parainfluenza viruses and the hemagglutinin–neuraminidase of Sendai virus in a manner dependent on the sialic acid residues carried by the NCRs [83–85]. Moreover, in mice, NKp46 has been demonstrated to be critical for resistance to influenza infection [86]. Furthermore, there is increasing evidence for the implication of the three human NCRs in NK cell-mediated killing of cells infected by a variety of viruses, including Ebola or Marburg viruses [87], vaccinia virus [88] or HSV-1 [89].

Finally, human NK cells have been shown to express functional TLRs that may contribute to their recognition of pathogens or infected cells (reviewed in [10]).

Impacts of NK cell functions on infection outcome & their mode of action

In addition to their direct contribution to the control of pathogen replication, NK cells have more recently been demonstrated to exert immunoregulatory functions during infections, including the modulation of DCs and T-cell responses. In most of the cases studied, NK cell antimicrobial or immunoregulatory functions appear to be beneficial to the host by limiting the extent of the damage caused directly by the replication of the pathogen or indirectly by the responses of other immune cells. However, in certain infections, NK cells may, conversely, contribute to the disease. This diversity of NK cell functions is illustrated in Figure 1A and detailed below.

Protective effects

Direct antimicrobial effects—Natural killer cells have been demonstrated to play a major role in the control of the replication of a number of viruses early after infection in mice and, in most cases, this antimicrobial effect was mediated by their cytotoxic activity. NK cell-mediated killing almost always leads to the death of the target cell through apoptosis, although different signaling pathways are utilized depending on the receptors that are engaged on NK cells [90]. For example, beige mice [23] or mice deficient in perforin or granzymes [91–93] show higher viral titers early after challenge with MCMV, at a time when adaptive immune responses are not yet functional. Beige and perforin-deficient mice eventually succumb to the infection. Other mechanisms used by NK cells to kill target cells include Fas ligand–Fas interaction, as proposed for red blood cells infected by *Plasmodium falciparum* [94], or tumor

necrosis factor-related apoptosis-inducing ligand (TRAIL), as demonstrated for encephalomyocarditis virus (EMCV) infection [95] or for HBV-expressing hepatocytes [96]. Thus, NK cells are generally thought to control viral replication and prevent the development of severe disease, mostly through the killing of infected cells. However, evidence also exists for the ability of NK cells to inhibit pathogen replication through noncytolytic mechanisms that may preserve the integrity of infected cells [97,98]. The major contribution of NK cells to early IFN- γ production during a number of infections could contribute to this noncytolytic control of pathogens, for example, by enforcing cell-intrinsic antimicrobial defenses in monocytes/macrophages [99] or in hepatocytes [93]. This non-cytolytic antimicrobial activity may also be especially important in the control of pathogen replication in tissues endowed with vital functions that are characterized by an inherent resistance to perforin-dependent cytotoxic activity, such as the liver [100]. In this context, it is noteworthy that NK cell-dependent control of MCMV replication has been reported to rely mostly on perforin-mediated killing of infected cells in the spleen and on IFN- γ production in the liver [93], although this has since been disputed [92]. The liver is the major site of viral replication in mice acutely infected with MCMV, and NK cells are critical for the control of viral replication locally. However, interestingly, it has been demonstrated recently that the viruses produced in the liver do not disseminate to other anatomical sites and that this control of dissemination is independent of NK cells and adaptive immunity [101].

Immunoregulatory functions—In addition to their direct antimicrobial activity, NK cells exert a variety of immunoregulatory effects that can contribute to promoting health over disease during infections. For example, at high doses of MCMV inoculum, NK cells protect the stromal cells of the splenic white pulp from massive infection and destruction, which prevents the disruption of the architecture of the spleen and the consecutive development of a state of general immunodeficiency [102]. More generally, NK cells can promote the development of adaptive immunity by shaping the cytokine milieu generated early after infection and the responses of DCs at the time of T-cell priming. Specifically, through their release of TNF- α and their engagement in cell–cell contacts, NK cells can induce DC maturation *in vitro* [103–105]. *In vivo*, in response to the stimulation with adjuvants or to the injection of mature DCs, NK cells are rapidly recruited to lymphoid organs where they produce IFN- γ early and thus promote the induction of Th1 responses [106–108].

Recently, efficient antiviral NK cell activity has been shown to accelerate the development of CD8 T-cell responses to MCMV infection, even at low doses of viral inoculum, by preventing excessive production of type I IFNs and other innate cytokines and their downstream toxic effects on CD8 α^+ DCs and CD8 T cells themselves [109,110]. During *T. gondii* infection, NK cells promote efficient CD8 T-cell responses in the absence of CD4 T-cell help, in an IFN- γ -dependent manner [111]. NK cells also prevent the development of immunopathology through downmodulating the activation of other innate cells. In particular, LCMV [112] or MCMV [91] infections of mice deficient in perforin-mediated killing or those depleted of NK cells result in severe disease characterized by the accumulation of highly activated TNF- α -producing macrophages that have engulfed erythrocytes. These models are strongly reminiscent of the hemophagocytic lymphohistiocytosis (HLH) syndrome in humans, which is in part caused by genetic deficiencies in perforin-dependent killing and/or infections with herpes viruses or *Leishmania* [33]. Interestingly, NK cells are not involved in the control of viral replication in LCMV infection [3], demonstrating that beneficial immunoregulatory effects of NK cells can be uncoupled from their antimicrobial activity.

Deleterious effects

Implication of NK cell-mediated destruction of infected cells in pathology—

While pathogen killing is often crucial to prevent the development of disease, the destruction

of infected cells by NK cells can be detrimental to the host if it contributes to organ failure as proposed, for example, in the case of liver damage in the transgenic mouse model of HBV infection [96,113]. In chronic HCV infection, a positive correlation has been reported between liver injury and the frequencies of activating NK receptors, further suggesting a potential deleterious role of NK cells in the development of liver cirrhosis [44].

Implication of NK cell-mediated inflammation in pathology—Another mechanism through which NK cells may cause deleterious effects to the host is their contribution to inflammation. NK cells can produce high levels of IFN- γ and other cytokines and chemokines during infections, as exemplified in the MCMV model [64]. In response to *T. gondii* infection in C57BL/6 mice, NK cells contribute to an overwhelming inflammatory response in the liver and the intestine, which can cause the death of the animals through multiorgan failure. The knockout of CCR5 prevents NK cell recruitment to the liver and protects the mice from the hyperinflammation but leads to the loss of control of pathogen replication and to death [31]. The production of proinflammatory mediators by NK cells can contribute to the induction of a toxic shock, both directly and indirectly via the downstream recruitment of other immune cells that can cause damage to the inflamed organ, such as T cells. For example, in *P. berghei* infection, NK cells recruit activated T cells to the brain in an IFN- γ -dependent manner and promote the induction of cerebral malaria [114]. This detrimental effect can be prevented by NK cell depletion. However, this treatment also results in the loss of control over parasite replication and only delays death by a few days. In human CMV infection, it has been proposed that NK cells contribute to the development of chronic inflammatory vascular disease through their recruitment and activation by infected endothelial cells [115].

Deregulation of NK cell functions leading to massive killing of uninfected cells—Recently, a series of provocative papers has been published by Debré's group that implicate NK cells as mediators of the massive killing of uninfected CD4 T cells, which leads to the development of AIDS [116]. The authors have shown that NK cells from individuals chronically infected with HIV-1 express NKp44, and that CD4 T cells from these individuals express high levels of the NKp44 ligand (NKp44L). Furthermore, they showed that NK cells from infected individuals can kill autologous CD4 T cells in an NKp44-dependent manner, and suggested that a particular peptide of the gp41 glycoprotein of HIV-1, named 3S, is responsible for the induction of NKp44L on CD4 T cells.

Thus, NK cell activity can represent a double-edged sword that needs to be tightly regulated so that NK cells can contribute to the early control of the infectious agents and the induction of pathogen-specific adaptive immunity without causing irreversible damage to the host. This concept is further supported by population genetic evidence showing that KIR haplotypes are under balancing selection and are associated not only with resistance to infections but also with autoimmunity or immunopathology [117,118]. With this in mind, we discuss some of the basic requirements for the successful design of NK cell-based immunotherapies against infectious agents, and possible strategies to meet them, as recapitulated in Figure 1B. Some of these strategies are inspired in part from approaches used or proposed to harness NK cells for defense against cancer (reviewed in [119,120]).

Therapeutic modulation of NK cell functions to improve defense against infections

Restoration/enforcement of NK cell effector potential

In HIV-1 or HCV infections, combinations of antiviral drugs and of NK cell activating cytokines, such as IL-2, IL-15 or type I IFNs, are being tested to attempt to restore a normal balance between NK cell subsets and to restore/enforce NK cell effector potential [121–123].

Alternative approaches include protocols to activate DCs by systemic administration of TLR ligands. The rationale for their development is to promote the release of endogenous NK cell-activating cytokines in a better dose-, time- and space-controlled fashion in order to favor beneficial responses over detrimental side effects (reviewed in [65,66]). However, boosting NK cell effector potential is not enough to foster the control of infectious agents by NK cells, as promoting the specific recognition of infected cells by NK cells remains a critical determinate.

Promotion of the specific recognition of infected cells by NK cells

Different strategies can be envisioned that could promote the specific recognition of infected cells by NK cells. First, lowering the threshold required to trigger NK cell effector functions is conceivable, for example, by the administration of antagonistic antibodies to NK cell inhibitory receptors, as already demonstrated to be efficient for antitumoral defense in mice [124].

Second, the physical interaction between NK cells and infected cells and triggering of NK cell effector functions may be promoted by the administration of bispecific antibodies that recognize both a NK cell-specific activation receptor and a molecule specifically expressed at the surface of infected cells, through two distinct antigen-binding domains.

Third, by using feeder cells expressing ligands for NK cell activation receptors and membrane-bound IL-15, it could be possible to specifically and potently amplify primary NK cells *in vitro*, and to transduce them with recombinant chimeric activation receptors against targeted antigens, prior to reinfusion in the patients. Such strategies are already being developed with the specific perspective of improving the protocols of adoptive NK cell transfer for treatment of acute lymphoblastic leukemia, chronic lymphocytic leukemia or non-Hodgkin's lymphoma [125]. This approach could also be applied to prevent CMV primary infection or reactivation in the recipients of bone marrow or kidney transplants. An alternative strategy for anti-CMV immunotherapy of bone marrow recipients would be the adoptive transfer of semiallogeneic NK cell lines, as an adaptation of the haploidentical hematopoietic stem cell transplantation used for defense against acute myeloid leukemia [126]. The selection of donor NK cell lines lacking inhibitory receptors for the MHC class I alleles of the recipient or harboring an activation receptor associated with enhanced resistance to the infection should allow the specific recognition and killing of the recipient's cells infected with CMV.

Finally, once microbial ligands specifically recognized by NK cell activation receptors have been identified, *in vivo* expansion and activation of the corresponding subset of NK cells may be achieved through administration of a DC-targeted TLR-activating vector encoding the ligand. This strategy could allow the development of therapeutic vaccines with broader access and easier production under good manufacturing practices for clinical trials, compared with the adoptive transfer of *in vitro* manipulated autologous NK cells. In addition, it may also be included as a component of a preventive vaccine in healthy individuals to promote optimal induction of adaptive immunity.

Redirecting other immune cell specificity through ectopic transfer of NK cell recognition specificity for infectious agents

Recently, it has been demonstrated in the MCMV model that it is possible to harness the specificity of NK cell activation receptors in order to redirect the activity of other immune cells towards the targeted pathogen [127]. This strategy utilizes a gene therapy system that is based on the ectopic expression of NK cell activation receptors in CD8 T cells. The approach takes advantage of the similarities in the cytotoxic effector function of CD8 T cells and NK cells to combine complementary valuable features of these two cell types. These properties are the

amenability of CD8 T lymphocytes to genetic engineering and bulk culture and, in NK cells, their expression of germline-encoded receptors of broad specificity and their ability to recognize virus-infected cells, largely independently of the processes of antigen processing and presentation. In brief, the strategy consists of transducing activated polyclonal primary CD8 T-cell cultures with retroviral vectors encoding chimeric virus-specific activation receptors. These receptors are engineered by fusing the extracellular domain of a NK cell receptor of documented specificity against a given virus with the intracellular domain of the CD3 ζ chain, which will trigger the effector cytotoxic program of the CD8 T cells upon engagement of the receptor by its viral ligand.

Another strategy developed recently harnesses the antitumoral specificity of a NK cell receptor in order to recruit activated macrophages into antibody-dependent cellular cytotoxicity against cancer cells. It employs the generation of soluble chimeric proteins in which the constant region of human IgG₁ was fused to the extracellular portion of NKp30 [128]. This strategy may probably work for antiviral immunotherapy, given the ability of NKp44 and NKp46 to recognize certain viral hemagglutinins, as discussed earlier.

Prevention of triggering of activation receptors where NK cells contribute to infection-induced immunopathology

Concurrent to their observations that the gp41 3S peptide induces expression of NKp44L on CD4 T cells during HIV-1 infection and sensitizes them to NK lysis, and that anti-3S antibody titers in infected individuals correlate positively with CD4 T-cell counts, Vieillard and colleagues hypothesized that anti-3S immunization would induce blocking antibodies that would prevent the induction of NKp44L on CD4 T cells and their downstream elimination by NK cells. Proof of principle for this concept was demonstrated in macaques, as anti-3S immunization of the animals prevented the induction of NKp44L on CD4 T cells after challenge with a simian HIV recombinant virus and decreased CD4 T-cell apoptosis in peripheral blood and lymph nodes, while no significant differences in the viral loads of control versus vaccinated animals were observed [129]. Thus, these data suggest that deregulated expression of a ligand for a NK cell activation receptor contributes to HIV-induced pathology independently of the level of replication of the virus, such that anti-3S immunization may synergize with antiviral treatment to slow down disease progression in infected individuals. However, it should be noted that for CXCR4-tropic viruses, which are associated with a faster rate of progression towards AIDS, the extent of the pathology induced by the infection does not correlate with the titer of anti-3S antibodies in the simian HIV macaque model [130]. In addition, the observations reported by the Debré group are awaiting independent confirmation by other teams.

Expert commentary

An extensive body of work exists demonstrating *in vitro* with human cells or *in vivo* in the mouse that NK cells make critical contributions to the control of infections of many viruses, some intracellular bacteria and certain intracellular protozoan parasites. Our current knowledge regarding the role of NK cells in exemplary human or mouse infections is summarized in Table 1. The importance of NK cells in immunodefense against infectious agents is further supported by epidemiological studies in humans based on the investigation of primary NK cell deficiencies in patients with recurrent viral infections or on the correlation between the resistance to a given infection and the NK cell receptor/MHC loci haplotypes.

A growing body of evidence has accumulated in the last 5 years demonstrating a clear dissociation between the mechanisms that promote NK cell acquisition of effector potential and those that allow specific recognition of infected cells and consecutive spatially and temporally controlled triggering of cytotoxicity and cytokine release. Accessory cells, including DCs and monocytes/macrophages, have been shown to be critical for the former

process, not only by secreting activating cytokines, such as type I IFNs, IL-12, IL-15 and IL-18, but also through direct interactions with NK cells [65,66]. Different strategies of NK cell recognition of infected cells have been revealed. They include the sensing of the downmodulation of MHC class I or of other self molecules as initially predicted by the missing-self hypothesis, and the detection of self molecules induced by stress signals, or the direct recognition of PAMPs by NK cell activation receptors or TLRs. In addition to their antimicrobial activity, NK cells have also been demonstrated to exert immunoregulatory functions that could contribute to promoting the activation of adaptive immune defenses against the pathogen or limit the extent of the immunopathology induced by the infection. However, NK cell activation may not always benefit the host. Sometimes, NK cells may contribute to the immunopathology by compromising the functions of vital organs through the destruction of infected cells within or even through massive destruction of uninfected cells due to deregulated expression of the ligands for NK cell activation receptors, as proposed in HIV-1 infection.

The major advances in our understanding of the general biology of NK cells and of the mechanisms that naturally regulate their activity during infections have raised great hopes that innovative therapeutic strategies could be developed by harnessing NK cell antimicrobial functions. In combination with other treatments, it is believed that modulating NK cell activity will help to promote health over disease during life-threatening infections that cause major public-health problems worldwide, such as malaria, trypanosomiasis, leishmaniasis, HIV-1 or HCV infections. NK cell-based immunotherapies could also contribute to preventing CMV infection and its severe consequences in fetuses, newborns or the immunocompromised. Compared with previous clinical trials, which were based on the direct injection of cytokines, the injection of DCs or the *in vivo* activation of DCs should allow better tuning of NK cell activation for the purpose of promoting specific functions, as well as reducing toxic side effects. However, in order to enhance the control of infectious agents by NK cells, it is important to realize that boosting NK cell effector potential is not enough. It will also be critical to promote the specific recognition of infected cells by NK cells. This could be achieved by different means, most efficiently by combining the triggering of a NK cell activation receptor specifically upon recognition of the infected cells together with the downmodulation of NK cell inhibitory receptor signaling. Exciting data have been obtained in the last 5 years pertaining to the identification of the ligands of NK cell activation receptors, either directly encoded by pathogens or from host origin and induced at the surface of infected cells. However, most of these findings have been made with studies in mice and therefore need to be extended to humans. Indeed, it is sobering to realize how little we currently know on the antigenic specificity of most NK cell activation receptors, which may well represent the major limitation for the development of efficient NK cell-based immunotherapies against infectious agents.

Five-year view

A major challenge to be faced in the next 5 years will be the identification of the ligands or the design of potent specific agonists of the NK cell activation receptors. A number of teams are actively pursuing this research area. Viral hemagglutinins have been reported to bind and activate NKp46, and potentially NKp44. Whether these receptors can recognize other microbial ligands is unknown. To the best of our knowledge, no natural ligands have been identified so far for the activation of KIRs in humans. Recent data suggest that these receptors may recognize endogenous MHC class I molecules bound to pathogen-derived peptides. Although no formal proof of this has been obtained to date, it should be noted that peptide-dependent direct binding of activation KIRs to MHC class I molecules has been demonstrated [76]. Increasing our knowledge with regard to the mechanisms evolved by viruses or other pathogens to evade NK cells should also help to identify the ligands for activation receptors, and more generally, will

aid in our understanding of the molecular pathways that regulate NK cell activation and the triggering of their effector functions during infections.

The study of animal models with an immune system closer to that of the humans, such as nonhuman primates or humanized mice, is underway. These lines of investigation should significantly improve our understanding of the function of human NK cells in real time and space during infections, and enable us to design preclinical models to test novel NK cell-based immunotherapeutic strategies. However, considerable technological improvements are required for us to fully benefit from these animal models. Novel recipient mice engineered to more fully support the development of human NK cells from hematopoietic progenitors are needed. Additionally, new antibody reagents must be developed to enable the study of NK cells and their receptors in nonhuman primates. Finally, only recently have most researchers in the field become aware of the extent of NK cell diversity. While reports documenting the existence of CD56^{bright}CD16⁻ versus CD56^{dim}CD16⁺ NK cell subsets in human blood have been published for some time, the study of the distribution of these NK cell subsets in lymph nodes and other tissues [131], their ability to differentially interact with other immune cells [132,133] and their functional homology relationships with mouse NK cell subsets [134, 135], have only been initiated recently. These studies have already yielded important discoveries for improving our understanding of the orchestration of immune defenses against pathogens [131,136] and much more is expected to be learned in this area. Thus, many novel concepts and tools are currently being developed to deepen our understanding of a number of aspects of NK cell biology, which should bring major advances within the next 5 years to design innovative therapeutic strategies to efficiently harness these cells in the clinic for defense against infections.

Key issues

- Natural killer (NK) cells are cytotoxic lymphocytes of the innate immune system that can be universally defined in mammals as NKp46⁺CD3⁻ cells and can mediate a number of microbicidal or immunoregulatory functions.
- Preactivation of NK cells by cytokines and membrane interactions with dendritic cells or monocytes/macrophages is required for the expression of their effector machinery, including cytotoxic granules and IFN- γ .
- The triggering of NK cell functions is finely tuned by a balance between activation and inhibitory signals. Most NK cell inhibitory receptors monitor self MHC class I molecules expression at the cell surface and contribute to a tonic inhibition of NK cell functions by normal cells. Different NK cell activation receptors can recognize different types of ligands, including stress-induced self molecules, pathogen-associated molecular patterns and, perhaps, MHC class I molecules associated with microbial components.
- Because of the broad spectrum of their recognition system, NK cells can respond to a large variety of intracellular pathogens, including viruses, bacteria and protozoa.
- NK cells are deregulated in a number of human infections, to the extent that they have lost their antimicrobial activity or can even take part to the development of an immunopathology.
- The modulation of NK cell functions is a promising strategy for the design of innovative therapies against a number of infectious diseases.
- NK cell activity is a double-edge sword that needs to be carefully balanced to contribute to the early control of the infectious agents and to the induction of

adaptive immunity without causing the failure of vital organs or other irreversible damage to the host.

- Future directions to improve our knowledge of NK cell biology for application to the clinic include:
 - The deciphering of the role of NK cell activation receptors in the resistance to infectious agents;
 - The identification of the natural ligands of NK cell activation receptors;
 - The design of synthetic agonists of NK cell activation receptors;
 - The deciphering of the specific functions of NK cell subsets.

Acknowledgements

The authors would like to thank all the past and present members of our laboratories for their contribution to the studies on innate immune defense against viral infections. Owing to space limitations, certain studies could not be quoted. We apologize to colleagues for such omissions.

Supported by CNRS to M Dalod, Ministère de l'Enseignement Supérieur et de la Recherche to N Zucchini, Institutional grants to the Centre d'Immunologie de Marseille-Luminy, and grants by the NIH to M Altfeld.

References

Papers of special note have been highlighted as:

• of interest

•• of considerable interest

1. McDermott MF, Tschopp J. From inflammasomes to fevers, crystals and hypertension: how basic research explains inflammatory diseases. *Trends Mol Med* 2007;13(9):381–388. [PubMed: 17822957]
2. Santoli D, Trinchieri G, Lief FS. Cell-mediated cytotoxicity against virus-infected target cells in humans. I. Characterization of the effector lymphocyte. *J Immunol* 1978;121(2):526–531. [PubMed: 681748]
3. Bukowski JF, Woda BA, Habu S, Okumura K, Welsh RM. Natural killer cell depletion enhances virus synthesis and virus-induced hepatitis *in vivo*. *J Immunol* 1983;131(3):1531–1538. [PubMed: 6309965]
4. Biron CA, Byron KS, Sullivan JL. Severe herpesvirus infections in an adolescent without natural killer cells. *N Engl J Med* 1989;320(26):1731–1735. [PubMed: 2543925]
5. Lodoen MB, Lanier LL. Natural killer cells as an initial defense against pathogens. *Curr Opin Immunol* 2006;18(4):391–398. [PubMed: 16765573] • Recent review on the interactions between natural killer (NK) cells and pathogens.
6. Huntington ND, Voshchenrich CA, Di Santo JP. Developmental pathways that generate natural-killer-cell diversity in mice and humans. *Nat Rev Immunol* 2007;7(9):703–714. [PubMed: 17717540]
7. Walzer T, Blery M, Chaix J, et al. Identification, activation, and selective *in vivo* ablation of mouse NK cells via NKp46. *Proc Natl Acad Sci USA* 2007;104(9):3384–3389. [PubMed: 17360655]
8. Cooper MA, Fehniger TA, Caligiuri MA. The biology of human natural killer-cell subsets. *Trends Immunol* 2001;22(11):633–640. [PubMed: 11698225]
9. Fehniger TA, Cooper MA, Nuovo GJ, et al. CD56^{bright} natural killer cells are present in human lymph nodes and are activated by T cell-derived IL-2: a potential new link between adaptive and innate immunity. *Blood* 2003;101(8):3052–3057. [PubMed: 12480696]
10. Lauzon NM, Mian F, Ashkar AA. Toll-like receptors, natural killer cells and innate immunity. *Adv Exp Med Biol* 2007;598:1–11. [PubMed: 17892200]
11. Lanier LL. NK cell recognition. *Annu Rev Immunol* 2005;23:225–274. [PubMed: 15771571]

12. Eagle RA, Trowsdale J. Promiscuity and the single receptor: NKG2D. *Nat Rev Immunol* 2007;7(9): 737–744. [PubMed: 17673918]
13. Jinushi M, Takehara T, Kanto T, et al. Critical role of MHC class I-related chain A and B expression on IFN- α -stimulated dendritic cells in NK cell activation: impairment in chronic hepatitis C virus infection. *J Immunol* 2003;170(3):1249–1256. [PubMed: 12538683]
14. Lee SH, Miyagi T, Biron CA. Keeping NK cells in highly regulated antiviral warfare. *Trends Immunol* 2007;28(6):252–259. [PubMed: 17466596]
15. Korbel DS, Finney OC, Riley EM. Natural killer cells and innate immunity to protozoan pathogens. *Int J Parasitol* 2004;34(13–14):1517–1528. [PubMed: 15582528]
16. Small CL, McCormick S, Gill N, et al. NK cells play a critical protective role in host defense against acute extracellular *Staphylococcus aureus* bacterial infection in the lung. *J Immunol* 2008;180(8): 5558–5568. [PubMed: 18390740]
17. Alter G, Altfield M. NK cell function in HIV-1 infection. *Curr Mol Med* 2006;6(6):621–629. [PubMed: 17022732]
18. Guma M, Angulo A, Vilches C, Gomez-Lozano N, Malats N, Lopez-Botet M. Imprint of human cytomegalovirus infection on the NK cell receptor repertoire. *Blood* 2004;104(12):3664–3671. [PubMed: 15304389]
19. Golden-Mason L, Madrigal-Estebas L, McGrath E, et al. Altered natural killer cell subset distributions in resolved and persistent hepatitis C virus infection following single source exposure. *Gut* 2008;57:1121–1128. [PubMed: 18372499]
20. Alter G, Suscovich TJ, Kleiman M, et al. Low perforin and elevated SHIP-1 expression is associated with functional anergy of natural killer cells in chronic HIV-1 infection. *AIDS* 2006;20(11):1549–1551. [PubMed: 16847410] • Characterization of molecular mechanisms contributing to the functional defects of NK cells in HIV-1-infected individuals.
21. Majumder B, Venkatachari NJ, O'Leary S, Ayyavoo V. Vpr positive human immunodeficiency virus (HIV-1) infection impairs NK cell function indirectly through cytokine dysregulation of infected target cells. *J Virol* 2008;82(14):7189–7200. [PubMed: 18417583]
22. Hycza MD, Kovacs C, Loutfy M, et al. Distinct transcriptional profiles in *ex vivo* CD4⁺ and CD8⁺ T cells are established early in human immunodeficiency virus type 1 infection and are characterized by a chronic interferon response as well as extensive transcriptional changes in CD8⁺ T cells. *J Virol* 2007;81(7):3477–3486. [PubMed: 17251300]
23. Shellam GR, Allan JE, Papadimitriou JM, Bancroft GJ. Increased susceptibility to cytomegalovirus infection in beige mutant mice. *Proc Natl Acad Sci USA* 1981;78(8):5104–5108. [PubMed: 6272291]
24. Stein-Streilein J, Guffee J. *In vivo* treatment of mice and hamsters with antibodies to asialo GM1 increases morbidity and mortality to pulmonary influenza infection. *J Immunol* 1986;136(4):1435–1441. [PubMed: 3944461]
25. Scalzo AA, Fitzgerald NA, Wallace CR, et al. The effect of the *Cmv-1* resistance gene, which is linked to the natural killer cell gene complex, is mediated by natural killer cells. *J Immunol* 1992;149(2): 581–589. [PubMed: 1378069]
26. Brown MG, Dokun AO, Heusel JW, et al. Vital involvement of a natural killer cell activation receptor in resistance to viral infection. *Science* 2001;292(5518):934–937. [PubMed: 11340207]
27. Ghiasi H, Cai S, Perng GC, Nesburn AB, Wechsler SL. The role of natural killer cells in protection of mice against death and corneal scarring following ocular HSV-1 infection. *Antiviral Res* 2000;45 (1):33–45. [PubMed: 10774588]
28. Thapa M, Kuziel WA, Carr DJ. Susceptibility of CCR5-deficient mice to genital herpes simplex virus type 2 is linked to NK cell mobilization. *J Virol* 2007;81(8):3704–3713. [PubMed: 17267483]
29. Paya CV, Patick AK, Leibson PJ, Rodriguez M. Role of natural killer cells as immune effectors in encephalitis and demyelination induced by Theiler's virus. *J Immunol* 1989;143(1):95–102. [PubMed: 2543704]
30. Hansen DS, D'Ombrain MC, Schofield L. The role of leukocytes bearing natural killer complex receptors and killer immunoglobulin-like receptors in the immunology of malaria. *Curr Opin Immunol* 2007;19(4):416–423. [PubMed: 17702559]
31. Khan IA, Thomas SY, Moretto MM, et al. CCR5 is essential for NK cell trafficking and host survival following *Toxoplasma gondii* infection. *PLoS Pathog* 2006;2(6):E49. [PubMed: 16789839]

32. Orange JS. Human natural killer cell deficiencies and susceptibility to infection. *Microbes Infect* 2002;4(15):1545–1558. [PubMed: 12505527]
33. Janka GE. Hemophagocytic syndromes. *Blood Rev* 2007;21(5):245–253. [PubMed: 17590250]
34. Scott-Algara D, Truong LX, Versmisse P, et al. Cutting edge: increased NK cell activity in HIV-1-exposed but uninfected Vietnamese intravascular drug users. *J Immunol* 2003;171(11):5663–5667. [PubMed: 14634071]• Reports that HIV-1-exposed uninfected individuals harbor enhanced NK cell activity that may contribute to their protection.
35. Ravet S, Scott-Algara D, Bonnet E, et al. Distinctive NK cell receptor repertoires sustain high-level constitutive NK cell activation in HIV-exposed uninfected individuals. *Blood* 2007;109(10):4296–4305. [PubMed: 17272507]• Reports that HIV-1-exposed uninfected individuals harbor enhanced NK cell activity that may contribute to their protection.
36. Boulet S, Sharafi S, Simic N, et al. Increased proportion of KIR3DS1 homozygotes in HIV-exposed uninfected individuals. *AIDS* 2008;22(5):595–599. [PubMed: 18317000]• Reports that HIV-1-exposed uninfected individuals harbor enhanced NK cell activity that may contribute to their protection.
37. Lee SH, Girard S, Macina D, et al. Susceptibility to mouse cytomegalovirus is associated with deletion of an activating natural killer cell receptor of the C-type lectin superfamily. *Nat Genet* 2001;28(1):42–45. [PubMed: 11326273]
38. Scalzo AA, Brown MG, Chu DT, Heusel JW, Yokoyama WM, Forbes CA. Development of intra-natural killer complex (NKC) recombinant and congenic mouse strains for mapping and functional analysis of NK cell regulatory loci. *Immunogenetics* 1999;49(3):238–241. [PubMed: 9914339]
39. Pereira RA, Scalzo A, Simmons A. Cutting edge: a NK complex-linked locus governs acute versus latent herpes simplex virus infection of neurons. *J Immunol* 2001;166(10):5869–5873. [PubMed: 11342599]
40. Hansen DS, Evans KJ, D'Ombrian MC, et al. The natural killer complex regulates severe malarial pathogenesis and influences acquired immune responses to *Plasmodium berghei* ANKA. *Infect Immun* 2005;73(4):2288–2297. [PubMed: 15784573]
41. Scalzo AA, Lyons PA, Fitzgerald NA, Forbes CA, Yokoyama WM, Shellam GR. Genetic mapping of *Cmv1* in the region of mouse chromosome 6 encoding the NK gene complex-associated loci *Ly49* and *musNKR-P1*. *Genomics* 1995;27(3):435–441. [PubMed: 7558024]
42. Khakoo SI, Carrington M. KIR and disease: a model system or system of models? *Immunol Rev* 2006;214:186–201. [PubMed: 17100885]•• Demonstrates epistatic interactions between the polymorphisms for NK cell receptors and MHC class I alleles for protection against infections.
43. Martin MP, Qi Y, Gao X, et al. Innate partnership of HLA-B and KIR3DL1 subtypes against HIV-1. *Nat Genet* 2007;39(6):733–740. [PubMed: 17496894]•• Demonstrates epistatic interactions between the polymorphisms for NK cell receptors and MHC class I alleles for protection against infections.
44. Paladino N, Flores AC, Marcos CY, et al. Increased frequencies of activating natural killer receptors are associated with liver injury in individuals who do not eliminate hepatitis C virus. *Tissue Antigens* 2007;69(Suppl 1):109–111. [PubMed: 17445180]
45. Estefania E, Gomez-Lozano N, Portero F, et al. Influence of *KIR* gene diversity on the course of HSV-1 infection: resistance to the disease is associated with the absence of KIR2DL2 and KIR2DS2. *Tissue Antigens* 2007;70(1):34–41. [PubMed: 17559579]
46. Ahlenstiel G, Martin MP, Gao X, Carrington M, Rehermann B. Distinct KIR/HLA compound genotypes affect the kinetics of human antiviral natural killer cell responses. *J Clin Invest* 2008;118(3):1017–1026. [PubMed: 18246204]
47. Alter G, Martin MP, Teigen N, et al. Differential natural killer cell-mediated inhibition of HIV-1 replication based on distinct KIR/HLA subtypes. *J Exp Med* 2007;204(12):3027–3036. [PubMed: 18025129]•• Demonstrates that the activation receptor KIR3DS1 allows efficient NK cell killing of autologous HIV-1-infected CD4 T cells expressing the putative ligand HLA-B Bw4-80I.
48. Long BR, Ndhlovu LC, Oksenberg JR, et al. Conferral of enhanced natural killer cell function by KIR3DS1 in early human immunodeficiency virus type 1 infection. *J Virol* 2008;82(10):4785–4792. [PubMed: 18305035]

49. Jonjic S, Babic M, Polic B, Krmpotic A. Immune evasion of natural killer cells by viruses. *Curr Opin Immunol* 2008;20:30–38. [PubMed: 18206359]• Up-to-date didactic review on the various mechanisms evolved by viruses to escape NK cell immune responses.
50. Chapgier A, Wynn RF, Jouanguy E, et al. Human complete Stat-1 deficiency is associated with defective type I and II IFN responses *in vitro* but immunity to some low virulence viruses *in vivo*. *J Immunol* 2006;176(8):5078–5083. [PubMed: 16585605]
51. Nguyen KB, Salazar-Mather TP, Dalod MY, et al. Coordinated and distinct roles for IFN- α β , IL-12, and IL-15 regulation of NK cell responses to viral infection. *J Immunol* 2002;169(8):4279–4287. [PubMed: 12370359]
52. Zucchini N, Bessou G, Robbins SH, et al. Individual plasmacytoid dendritic cells are major contributors to the production of multiple innate cytokines in an organ-specific manner during viral infection. *Int Immunol* 2008;20(1):45–56. [PubMed: 18000008]
53. Schneider K, Loewendorf A, De Trez C, et al. Lymphotoxin-mediated crosstalk between B cells and splenic stroma promotes the initial type I interferon response to cytomegalovirus. *Cell Host Microbe* 2008;3(2):67–76. [PubMed: 18312841]
54. Martinez J, Huang X, Yang Y. Direct action of type I IFN on NK cells is required for their activation in response to vaccinia viral infection *in vivo*. *J Immunol* 2008;180(3):1592–1597. [PubMed: 18209055]• First rigorous demonstration of the requirement for a direct action of type I interferon on NK cells during a viral infection *in vivo* for the promotion of efficient antiviral activity.
55. Lucas M, Schachterle W, Oberle K, Aichele P, Diefenbach A. Dendritic cells prime natural killer cells by trans-presenting interleukin 15. *Immunity* 2007;26(4):503–517. [PubMed: 17398124]• Demonstrates that, during pathogen-like challenges, DCs promote the acquisition of effector potential by NK cells and their survival through cross-presentation of IL-15.
56. Mortier E, Woo T, Advincula R, Gozalo S, Ma A. IL-15R α chaperones IL-15 to stable dendritic cell membrane complexes that activate NK cells via trans presentation. *J Exp Med* 2008;205(5):1213–1225. [PubMed: 18458113]• Demonstrates that, during pathogen-like challenges, DCs promote the acquisition of effector potential by NK cells and their survival through cross-presentation of IL-15.
57. Brilot F, Strowig T, Roberts SM, Arrey F, Munz C. NK cell survival mediated through the regulatory synapse with human DCs requires IL-15R α . *J Clin Invest* 2007;117(11):3316–3329. [PubMed: 17948125]• Demonstrates that, during pathogen-like challenges, DCs promote the acquisition of effector potential by NK cells and their survival through cross-presentation of IL-15.
58. Baratin M, Roetyncck S, Lepolard C, et al. Natural killer cell and macrophage cooperation in MyD88-dependent innate responses to *Plasmodium falciparum*. *Proc Natl Acad Sci USA* 2005;102(41):14747–14752. [PubMed: 16203971]
59. French AR, Holroyd EB, Yang L, Kim S, Yokoyama WM. IL-18 acts synergistically with IL-15 in stimulating natural killer cell proliferation. *Cytokine* 2006;35(5–6):229–234. [PubMed: 17052916]
60. Liu B, Mori I, Hossain MJ, Dong L, Takeda K, Kimura Y. Interleukin-18 improves the early defence system against influenza virus infection by augmenting natural killer cell-mediated cytotoxicity. *J Gen Virol* 2004;85(Pt 2):423–428. [PubMed: 14769900]
61. Schleicher U, Liese J, Knippertz I, et al. NK cell activation in visceral leishmaniasis requires TLR9, myeloid DCs, and IL-12, but is independent of plasmacytoid DCs. *J Exp Med* 2007;204(4):893–906. [PubMed: 17389237]
62. Newman KC, Korbel DS, Hafalla JC, Riley EM. Cross-talk with myeloid accessory cells regulates human natural killer cell interferon- γ responses to malaria. *PLoS Pathog* 2006;2(12):E118. [PubMed: 17154717]
63. Chiesa S, Mingueneau M, Fuseri N, et al. Multiplicity and plasticity of natural killer cell signaling pathways. *Blood* 2006;107(6):2364–2372. [PubMed: 16291591]
64. Dorner BG, Smith HR, French AR, et al. Coordinate expression of cytokines and chemokines by NK cells during murine cytomegalovirus infection. *J Immunol* 2004;172(5):3119–3131. [PubMed: 14978118]
65. Walzer T, Dalod M, Vivier E, Zitvogel L. Natural killer cell-dendritic cell crosstalk in the initiation of immune responses. *Expert Opin Biol Ther* 2005;5(Suppl 1):S49–S59. [PubMed: 16187940]
66. Newman KC, Riley EM. Whatever turns you on: accessory-cell-dependent activation of NK cells by pathogens. *Nat Rev Immunol* 2007;7(4):279–291. [PubMed: 17380157]

67. Fehniger TA, Cai SF, Cao X, et al. Acquisition of murine NK cell cytotoxicity requires the translation of a pre-existing pool of granzyme B and perforin mRNAs. *Immunity* 2007;26(6):798–811. [PubMed: 17540585]•• Demonstrates that, compared with naïve CD8 T cells, resting NK cells are already prone for cytotoxicity since they constitutively express mRNA for perforin and granzymes, but that terminal acquisition of this effector potential requires the activation by innate cytokines to remove a post-translational block in effector molecule production.
68. Salazar-Mather TP, Orange JS, Biron CA. Early murine cytomegalovirus (MCMV) infection induces liver natural killer (NK) cell inflammation and protection through macrophage inflammatory protein 1 α (MIP-1 α)-dependent pathways. *J Exp Med* 1998;187(1):1–14. [PubMed: 9419206]
69. Gregoire C, Chasson L, Luci C, et al. The trafficking of natural killer cells. *Immunol Rev* 2007;220:169–182. [PubMed: 17979846]
70. Karre K. Natural killer cell recognition of missing self. *Nat Immunol* 2008;9(5):477–480. [PubMed: 18425103]
71. Voigt S, Mesci A, Ettinger J, et al. Cytomegalovirus evasion of innate immunity by subversion of the NKR-P1B:Clr-b missing-self axis. *Immunity* 2007;26(5):617–627. [PubMed: 17462921]
72. Le Maux Chansac B, Moretta A, Vergnon I, et al. NK cells infiltrating a MHC class I-deficient lung adenocarcinoma display impaired cytotoxic activity toward autologous tumor cells associated with altered NK cell-triggering receptors. *J Immunol* 2005;175(9):5790–5798. [PubMed: 16237071]
73. Carbone E, Neri P, Mesuraca M, et al. HLA class I, NKG2D, and natural cytotoxicity receptors regulate multiple myeloma cell recognition by natural killer cells. *Blood* 2005;105(1):251–258. [PubMed: 15328155]
74. Gasser S, Orsulic S, Brown EJ, Raulet DH. The DNA damage pathway regulates innate immune system ligands of the NKG2D receptor. *Nature* 2005;436(7054):1186–1190. [PubMed: 15995699]
75. Desrosiers MP, Kielczewska A, Loredó-Ostí JC, et al. Epistasis between mouse Klra and major histocompatibility complex class I loci is associated with a new mechanism of natural killer cell-mediated innate resistance to cytomegalovirus infection. *Nat Genet* 2005;37(6):593–599. [PubMed: 15895081]•• Demonstration of an epistatic interaction between the polymorphisms for a NK cell activation receptor and MHC class I alleles for protection against a viral infection.
76. Stewart CA, Laugier-Anfossi F, Vely F, et al. Recognition of peptide-MHC class I complexes by activating killer immunoglobulin-like receptors. *Proc Natl Acad Sci USA* 2005;102(37):13224–13229. [PubMed: 16141329]•• Demonstrates that both inhibitory and activating NK cell receptors can directly bind MHC class I molecules and that the affinity for this interaction is peptide- and allele-dependent.
77. Hansasuta P, Dong T, Thananchai H, et al. Recognition of HLA-A3 and HLA-A11 by KIR3DL2 is peptide-specific. *Eur J Immunol* 2004;34(6):1673–1679. [PubMed: 15162437]•• Demonstrates that both inhibitory and activating NK cell receptors can directly bind MHC class I molecules and that the affinity for this interaction is peptide- and allele-dependent.
78. Thananchai H, Gillespie G, Martin MP, et al. Cutting edge: allele-specific and peptide-dependent interactions between KIR3DL1 and HLA-A and HLA-B. *J Immunol* 2007;178(1):33–37. [PubMed: 17182537]•• Demonstrates that both inhibitory and activating NK cell receptors can directly bind MHC class I molecules and that the affinity for this interaction is peptide- and allele-dependent.
79. Arase H, Mocarski ES, Campbell AE, Hill AB, Lanier LL. Direct recognition of cytomegalovirus by activating and inhibitory NK cell receptors. *Science* 2002;296(5571):1323–1326. [PubMed: 11950999]
80. Smith HR, Heusel JW, Mehta IK, et al. Recognition of a virus-encoded ligand by a natural killer cell activation receptor. *Proc Natl Acad Sci USA* 2002;99(13):8826–8831. [PubMed: 12060703]
81. Bubic I, Wagner M, Krmpotić A, et al. Gain of virulence caused by loss of a gene in murine cytomegalovirus. *J Virol* 2004;78(14):7536–7544. [PubMed: 15220428]
82. Arase H, Lanier LL. Specific recognition of virus-infected cells by paired NK receptors. *Rev Med Virol* 2004;14(2):83–93. [PubMed: 15027001]
83. Ho JW, Hershkowitz O, Peiris M, et al. H5-type influenza virus hemagglutinin is functionally recognized by the natural killer-activation receptor NKp44. *J Virol* 2008;82(4):2028–2032. [PubMed: 18077718]

84. Mandelboim O, Lieberman N, Lev M, et al. Recognition of haemagglutinins on virus-infected cells by NKp46 activates lysis by human NK cells. *Nature* 2001;409(6823):1055–1060. [PubMed: 11234016]
85. Arnon TI, Achdout H, Lieberman N, et al. The mechanisms controlling the recognition of tumor- and virus-infected cells by NKp46. *Blood* 2004;103(2):664–672. [PubMed: 14504081]
86. Gazit R, Gruda R, Elboim M, et al. Lethal influenza infection in the absence of the natural killer cell receptor gene. *Ncr1 Nat Immunol* 2006;7:517–523. • Demonstration that NKp46 is critical to promote enhanced resistance to influenza infection in mice.
87. Fuller CL, Ruthel G, Warfield KL, et al. NKp30-dependent cytolysis of filovirus-infected human dendritic cells. *Cell Microbiol* 2007;9(4):962–976. [PubMed: 17381429]
88. Chisholm SE, Reyburn HT. Recognition of vaccinia virus-infected cells by human natural killer cells depends on natural cytotoxicity receptors. *J Virol* 2006;80(5):2225–2233. [PubMed: 16474130]
89. Chisholm SE, Howard K, Gomez MV, Reyburn HT. Expression of ICP0 is sufficient to trigger natural killer cell recognition of herpes simplex virus-infected cells by natural cytotoxicity receptors. *J Infect Dis* 2007;195(8):1160–1168. [PubMed: 17357052]
90. Smyth MJ, Cretney E, Kelly JM, et al. Activation of NK cell cytotoxicity. *Mol Immunol* 2005;42(4):501–510. [PubMed: 15607806]
91. van Dommelen SL, Sumaria N, Schreiber RD, Scalzo AA, Smyth MJ, Degli-Esposti MA. Perforin and granzymes have distinct roles in defensive immunity and immunopathology. *Immunity* 2006;25(5):835–848. [PubMed: 17088087] • Demonstrates that NK cells exert protective immunoregulatory functions during two different viral infections to prevent macrophage hyperactivation and the development of a hemophagocytic lymphohistiocytosis-like syndrome.
92. Loh J, Chu DT, O'Guin AK, Yokoyama WM, Virgin HW. Natural killer cells utilize both perforin and γ interferon to regulate murine cytomegalovirus infection in the spleen and liver. *J Virol* 2005;79(1):661–667. [PubMed: 15596864]
93. Tay CH, Welsh RM. Distinct organ-dependent mechanisms for the control of murine cytomegalovirus infection by natural killer cells. *J Virol* 1997;71(1):267–275. [PubMed: 8985346]
94. Mavoungou E, Luty AJ, Kremsner PG. Natural killer (NK) cell-mediated cytolysis of *Plasmodium falciparum*-infected human red blood cells *in vitro*. *Eur Cytokine Netw* 2003;14(3):134–142. [PubMed: 14656686]
95. Sato K, Hida S, Takayanagi H, et al. Antiviral response by natural killer cells through *TRAIL* gene induction by IFN- α/β . *Eur J Immunol* 2001;31(11):3138–3146. [PubMed: 11745330]
96. Dunn C, Brunetto M, Reynolds G, et al. Cytokines induced during chronic hepatitis B virus infection promote a pathway for NK cell-mediated liver damage. *J Exp Med* 2007;204(3):667–680. [PubMed: 17353365] • Demonstrates that NK cells may not only develop protective functions but could also contribute to the immunopathology during various infections.
97. Iversen AC, Norris PS, Ware CF, Benedict CA. Human NK cells inhibit cytomegalovirus replication through a noncytolytic mechanism involving lymphotoxin-dependent induction of IFN- β . *J Immunol* 2005;175(11):7568–7574. [PubMed: 16301666] • Molecular dissection of the noncytolytic function of NK cells involved in the control of cytomegalovirus replication in target cells.
98. Bernstein HB, Kinter AL, Jackson R, Fauci AS. Neonatal natural killer cells produce chemokines and suppress HIV replication *in vitro*. *AIDS Res Hum Retroviruses* 2004;20(11):1189–1195. [PubMed: 15588341]
99. Presti RM, Popkin DL, Connick M, Paetzold S, Virgin HW. Novel cell type-specific antiviral mechanism of interferon γ action in macrophages. *J Exp Med* 2001;193(4):483–496. [PubMed: 11181700]
100. Willberg CB, Ward SM, Clayton RF, et al. Protection of hepatocytes from cytotoxic T cell mediated killing by interferon- α . *PLoS ONE* 2007;2(8):E791. [PubMed: 17726521]
101. Sacher T, Podlech J, Mohr CA, et al. The major virus-producing cell type during murine cytomegalovirus infection, the hepatocyte, is not the source of virus dissemination in the host. *Cell Host Microbe* 2008;3(4):263–272. [PubMed: 18407069]
102. Bekiaris V, Timoshenko O, Hou TZ, et al. Ly49H⁺ NK cells migrate to and protect splenic white pulp stroma from murine cytomegalovirus infection. *J Immunol* 2008;180(10):6768–6776. [PubMed: 18453597]

103. Gerosa F, Baldani-Guerra B, Nisii C, Marchesini V, Carra G, Trinchieri G. Reciprocal activating interaction between natural killer cells and dendritic cells. *J Exp Med* 2002;195(3):327–333. [PubMed: 11828007]
104. Gerosa F, Gobbi A, Zorzi P, et al. The reciprocal interaction of NK cells with plasmacytoid or myeloid dendritic cells profoundly affects innate resistance functions. *J Immunol* 2005;174(2):727–734. [PubMed: 15634892]
105. Piccioli D, Sbrana S, Melandri E, Valiante NM. Contact-dependent stimulation and inhibition of dendritic cells by natural killer cells. *J Exp Med* 2002;195(3):335–341. [PubMed: 11828008]
106. Martin-Fontecha A, Thomsen LL, Brett S, et al. Induced recruitment of NK cells to lymph nodes provides IFN- γ for T(H)1 priming. *Nat Immunol* 2004;5(12):1260–1265. [PubMed: 15531883]
107. Morandi B, Bougras G, Muller WA, Ferlazzo G, Munz C. NK cells of human secondary lymphoid tissues enhance T cell polarization via IFN- γ secretion. *Eur J Immunol* 2006;36(9):2394–2400. [PubMed: 16917961]
108. Laouar Y, Sutterwala FS, Gorelik L, Flavell RA. Transforming growth factor- β controls T helper type 1 cell development through regulation of natural killer cell interferon- γ . *Nat Immunol* 2005;6(6):600–607. [PubMed: 15852008]
109. Andrews DM, Andoniu CE, Scalzo AA, et al. Cross-talk between dendritic cells and natural killer cells in viral infection. *Mol Immunol* 2005;42(4):547–555. [PubMed: 15607812]
110. Robbins SH, Bessou G, Cornillon A, et al. Natural killer cells promote early CD8 T cell responses against cytomegalovirus. *PLoS Pathog* 2007;3(8):E123. [PubMed: 17722980]•• Demonstrates that NK cell activity can contribute to promote the induction of early, protective, CD8 T-cell responses to intracellular pathogens.
111. Goldszmid RS, Bafica A, Jankovic D, et al. TAP-1 indirectly regulates CD4⁺ T cell priming in *Toxoplasma gondii* infection by controlling NK cell IFN- γ production. *J Exp Med* 2007;204(11):2591–2602. [PubMed: 17923502]•• Demonstrates that NK cell activity can contribute to promote the induction of early, protective, CD8 T-cell responses to intracellular pathogens.
112. Crozat K, Hoebe K, Ugolini S, et al. Jinx, an MCMV susceptibility phenotype caused by disruption of Unc13d: a mouse model of type 3 familial hemophagocytic lymphohistiocytosis. *J Exp Med* 2007;204(4):853–863. [PubMed: 17420270]•• Demonstrates that NK cells exert protective immunoregulatory functions during two different viral infections to prevent macrophage hyperactivation and the development of a HLH-like syndrome.
113. Chen Y, Wei H, Sun R, Dong Z, Zhang J, Tian Z. Increased susceptibility to liver injury in hepatitis B virus transgenic mice involves NKG2D-ligand interaction and natural killer cells. *Hepatology* 2007;46(3):706–715. [PubMed: 17626270]
114. Hansen DS, Bernard NJ, Nie CQ, Schofield L. NK cells stimulate recruitment of CXCR3⁺ T cells to the brain during *Plasmodium berghei*-mediated cerebral malaria. *J Immunol* 2007;178(9):5779–5788. [PubMed: 17442962]• Demonstrates that NK cells may not only develop protective functions but could also contribute to the immunopathology during various infections.
115. Bolovan-Fritts CA, Spector SA. Endothelial damage from cytomegalovirus-specific host immune response can be prevented by targeted disruption of fractalkine–CX3CR1 interaction. *Blood* 2008;111(1):175–182. [PubMed: 17895402]• Demonstrates that NK cells may not only develop protective functions but could also contribute to the immunopathology during various infections.
116. Vieillard V, Strominger JL, Debre P. NK cytotoxicity against CD4⁺ T cells during HIV-1 infection: a gp41 peptide induces the expression of an Nkp44 ligand. *Proc Natl Acad Sci USA* 2005;102(31):10981–10986. [PubMed: 16046540]•• Belongs to a series of provocative papers from the group of Debré suggesting that NK cells bear a major contribution to HIV-1-induced immunopathology through massive killing of uninfected CD4 T cells and that a vaccination strategy could be designed to contribute to slow disease progression by preventing this phenomenon.
117. Kulkarni S, Martin MP, Carrington M. The yin and yang of HLA and KIR in human disease. *Semin Immunol*. 2008;10.1016/j.smim.2008.06.003Epub ahead of print
118. Parham P. MHC class I molecules and KIRs in human history, health and survival. *Nat Rev Immunol* 2005;5(3):201–214. [PubMed: 15719024]
119. Ljunggren HG, Malmberg KJ. Prospects for the use of NK cells in immunotherapy of human cancer. *Nat Rev Immunol* 2007;7(5):329–339. [PubMed: 17438573]

120. Terme M, Ullrich E, Delahaye NF, Chaput N, Zitvogel L. Natural killer cell-directed therapies: moving from unexpected results to successful strategies. *Nat Immunol* 2008;9(5):486–494. [PubMed: 18425105]
121. d'Ettorre G, Andreotti M, Carnevalini M, et al. Interleukin-15 enhances the secretion of IFN- γ and CC chemokines by natural killer cells from HIV viremic and aviremic patients. *Immunol Lett* 2006;103(2):192–195. [PubMed: 16289324]
122. Rodriguez AR, Arulanandam BP, Hodara VL, et al. Influence of interleukin-15 on CD8⁺ natural killer cells in human immunodeficiency virus type 1-infected chimpanzees. *J Gen Virol* 2007;88 (Pt 2):641–651. [PubMed: 17251583]
123. Michaelsson J, Long BR, Loo CP, et al. Immune reconstitution of CD56^{dim} NK cells in individuals with primary HIV-1 infection treated with interleukin-2. *J Infect Dis* 2008;197(1):117–125. [PubMed: 18171294]
124. Koh CY, Blazar BR, George T, et al. Augmentation of antitumor effects by NK cell inhibitory receptor blockade *in vitro* and *in vivo*. *Blood* 2001;97(10):3132–3137. [PubMed: 11342440]
125. Imai C, Iwamoto S, Campana D. Genetic modification of primary natural killer cells overcomes inhibitory signals and induces specific killing of leukemic cells. *Blood* 2005;106(1):376–383. [PubMed: 15755898]
126. Ruggeri L, Capanni M, Urbani E, et al. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. *Science* 2002;295(5562):2097–2100. [PubMed: 11896281]
127. Iizuka K, Nakajima C, Iizuka YM, et al. Protection from lethal infection by adoptive transfer of CD8 T cells genetically engineered to express virus-specific innate immune receptor. *J Immunol* 2007;179(2):1122–1128. [PubMed: 17617605]
128. Arnon TI, Markel G, Bar-Ilan A, et al. Harnessing soluble NK cell killer receptors for the generation of novel cancer immune therapy. *PLoS ONE* 2008;3(5):E2150. [PubMed: 18478075]
129. Vieillard V, Le Grand R, Dausset J, Debre P. A vaccine strategy against AIDS: an HIV gp41 peptide immunization prevents NKp44L expression and CD4⁺ T cell depletion in SHIV-infected macaques. *Proc Natl Acad Sci USA* 2008;105(6):2100–2104. [PubMed: 18234855]•• Belongs to a series of provocative papers from the group of Debré suggesting that NK cells bear a major contribution to HIV-1-induced immunopathology through massive killing of uninfected CD4 T cells and that a vaccination strategy could be designed to contribute to slow disease progression by preventing this phenomenon.
130. Vieillard V, Habib RE, Brochard P, et al. CCR5 or CXCR4 use influences the relationship between CD4 cell depletion, NKp44L expression and NK cytotoxicity in SHIV-infected macaques. *AIDS* 2008;22(2):185–192. [PubMed: 18097220]
131. Ferlazzo G, Thomas D, Lin SL, et al. The abundant NK cells in human secondary lymphoid tissues require activation to express killer cell Ig-like receptors and become cytolytic. *J Immunol* 2004;172 (3):1455–1462. [PubMed: 14734722]
132. Vitale M, Della Chiesa M, Carlomagno S, et al. The small subset of CD56^{bright}CD16-natural killer cells is selectively responsible for both cell proliferation and interferon- γ production upon interaction with dendritic cells. *Eur J Immunol* 2004;34(6):1715–1722. [PubMed: 15162442]
133. Mavilio D, Lombardo G, Kinter A, et al. Characterization of the defective interaction between a subset of natural killer cells and dendritic cells in HIV-1 infection. *J Exp Med* 2006;203(10):2339–2350. [PubMed: 17000867]
134. Vossen MT, Matmati M, Hertoghs KM, et al. CD27 defines phenotypically and functionally different human NK cell subsets. *J Immunol* 2008;180(6):3739–3745. [PubMed: 18322179]•• One of the first reports to propose the use of identical markers for the identification and comparison of mouse and human NK cell subsets.
135. Silva A, Andrews DM, Brooks AG, Smyth MJ, Hayakawa Y. Application of CD27 as a marker for distinguishing human NK cell subsets. *Int Immunol* 2008;20(4):625–630. [PubMed: 18326863]•• One of the first reports to propose the use of identical markers for the identification and comparison of mouse and human NK cell subsets.
136. Strowig T, Brilot F, Arrey F, et al. Tonsillar NK cells restrict B cell transformation by the Epstein-Barr virus via IFN- γ . *PLoS Pathog* 2008;4(2):E27. [PubMed: 18266470]• One of the first papers to analyze the antiviral functions of human NK cells from secondary lymphoid organs.

137. Adam SG, Caraux A, Fodil-Cornu N, et al. Cmv4, a new locus linked to the NK cell gene complex, controls innate resistance to cytomegalovirus in wild-derived mice. *J Immunol* 2006;176(9):5478–5485. [PubMed: 16622016]
138. Arnon TI, Lev M, Katz G, Chernobrov Y, Porgador A, Mandelboim O. Recognition of viral hemagglutinins by NKp44 but not by NKp30. *Eur J Immunol* 2001;31(9):2680–2689. [PubMed: 11536166]
139. Draghi M, Pashine A, Sanjanwala B, et al. NKp46 and NKG2D recognition of infected dendritic cells is necessary for NK cell activation in the human response to influenza infection. *J Immunol* 2007;178(5):2688–2698. [PubMed: 17312110]
140. Brooks CR, Elliott T, Parham P, Khakoo SI. The inhibitory receptor NKG2A determines lysis of vaccinia virus-infected autologous targets by NK cells. *J Immunol* 2006;176(2):1141–1147. [PubMed: 16434388]
141. Dalod M, Hamilton T, Salomon R, et al. Dendritic cell responses to early murine cytomegalovirus infection: subset functional specialization and differential regulation by interferon α/β . *J Exp Med* 2003;197(7):885–898. [PubMed: 12682109]
142. Krug A, French AR, Barchet W, et al. TLR9-dependent recognition of MCMV by IPC and DC generates coordinated cytokine responses that activate antiviral NK cell function. *Immunity* 2004;21(1):107–119. [PubMed: 15345224]
143. French AR, Pingel JT, Wagner M, et al. Escape of mutant double-stranded DNA virus from innate immune control. *Immunity* 2004;20(6):747–756. [PubMed: 15189739]• Demonstrates that strong NK cell pressure can lead to the selection of viral escape mutants *in vivo*.
144. Voigt V, Forbes CA, Tonkin JN, et al. Murine cytomegalovirus m157 mutation and variation leads to immune evasion of natural killer cells. *Proc Natl Acad Sci USA* 2003;100(23):13483–13488. [PubMed: 14597723]• Demonstrates that strong NK cell pressure can lead to the selection of viral escape mutants *in vivo*.
145. Alter G, Teigen N, Davis BT, et al. Sequential deregulation of NK cell subset distribution and function starting in acute HIV-1 infection. *Blood* 2005;106(10):3366–3369. [PubMed: 16002429]
146. Mavilio D, Lombardo G, Benjamin J, et al. Characterization of CD56⁺/CD16⁺ natural killer (NK) cells: a highly dysfunctional NK subset expanded in HIV-infected viremic individuals. *Proc Natl Acad Sci USA* 2005;102(8):2886–2891. [PubMed: 15699323]
147. Ward J, Bonaparte M, Sacks J, et al. HIV modulates the expression of ligands important in triggering natural killer cell cytotoxic responses on infected primary T-cell blasts. *Blood* 2007;110(4):1207–1214. [PubMed: 17513617]
148. Cohen GB, Gandhi RT, Davis DM, et al. The selective downregulation of class I major histocompatibility complex proteins by HIV-1 protects HIV-infected cells from NK cells. *Immunity* 1999;10(6):661–671. [PubMed: 10403641]
149. Achdout H, Arnon TI, Markel G, et al. Enhanced recognition of human NK receptors after influenza virus infection. *J Immunol* 2003;171(2):915–923. [PubMed: 12847262]
150. Noah DL, Twu KY, Krug RM. Cellular antiviral responses against influenza A virus are countered at the posttranscriptional level by the viral NS1A protein via its binding to a cellular protein required for the 3' end processing of cellular pre-mRNAs. *Virology* 2003;307(2):386–395. [PubMed: 12667806]
151. Kirwan S, Merriam D, Barsby N, McKinnon A, Burshtyn DN. Vaccinia virus modulation of natural killer cell function by direct infection. *Virology* 2006;347(1):75–87. [PubMed: 16387342]
152. Reading PC, Smith GL. Vaccinia virus interleukin-18-binding protein promotes virulence by reducing γ interferon production and natural killer and T-cell activity. *J Virol* 2003;77(18):9960–9968. [PubMed: 12941906]
153. Najjarro P, Traktman P, Lewis JA. Vaccinia virus blocks γ interferon signal transduction: viral Vh1 phosphatase reverses Stat1 activation. *J Virol* 2001;75(7):3185–3196. [PubMed: 11238845]
154. Su HC, Nguyen KB, Salazar-Mather TP, Ruzek MC, Dalod MY, Biron CA. NK cell functions restrain T cell responses during viral infections. *Eur J Immunol* 2001;31(10):3048–3055. [PubMed: 11592081]
155. Alter G, Teigen N, Ahern R, et al. Evolution of innate and adaptive effector cell functions during acute HIV-1 infection. *J Infect Dis* 2007;195(10):1452–1460. [PubMed: 17436225]

156. Mavoungou E, Held J, Mewono L, Kremsner PG. A Duffy binding-like domain is involved in the NKp30-mediated recognition of *Plasmodium falciparum*-parasitized erythrocytes by natural killer cells. *J Infect Dis* 2007;195(10):1521–1531. [PubMed: 17436233]
157. Baratin M, Roetynck S, Pouvelle B, et al. Dissection of the role of PfEMP1 and ICAM-1 in the sensing of *Plasmodium falciparum*-infected erythrocytes by natural killer cells. *PLoS ONE* 2007;2(2):E228. [PubMed: 17311092]
158. D'Ombain MC, Voss TS, Maier AG, et al. *Plasmodium falciparum* erythrocyte membrane protein-1 specifically suppresses early production of host interferon- γ . *Cell Host Microbe* 2007;2(2):130–138. [PubMed: 18005727]
159. Artavanis-Tsakonas K, Riley EM. Innate immune response to malaria: rapid induction of IFN- γ from human NK cells by live *Plasmodium falciparum*-infected erythrocytes. *J Immunol* 2002;169(6):2956–2963. [PubMed: 12218109]
160. Esin S, Batoni G, Counoupas C, et al. Direct binding of human NK cell natural cytotoxicity receptor NKp44 to the surfaces of mycobacteria and other bacteria. *Infect Immun* 2008;76(4):1719–1727. [PubMed: 18212080]
161. Vankayalapati R, Garg A, Porgador A, et al. Role of NK cell-activation receptors and their ligands in the lysis of mononuclear phagocytes infected with an intracellular bacterium. *J Immunol* 2005;175(7):4611–4617. [PubMed: 16177106]
162. Schierloh P, Yokobori N, Aleman M, et al. *Mycobacterium tuberculosis*-induced γ -interferon production by natural killer cells requires cross talk with antigen-presenting cells involving Toll-like receptors 2 and 4 and the mannose receptor in tuberculous pleurisy. *Infect Immun* 2007;75(11):5325–5337. [PubMed: 17709420]
163. Vankayalapati R, Klucar P, Wizel B, et al. NK cells regulate CD8⁺ T cell effector function in response to an intracellular pathogen. *J Immunol* 2004;172(1):130–137. [PubMed: 14688318]
164. Roy S, Barnes PF, Garg A, Wu S, Cosman D, Vankayalapati R. NK cells lyse T regulatory cells that expand in response to an intracellular pathogen. *J Immunol* 2008;180(3):1729–1736. [PubMed: 18209070]

Figure 1. Molecular and cellular mechanisms of natural killer cell antimicrobial activities and potential implications for the design of immunotherapeutic interventions against infective agents (A) Schematic representation of the mechanisms regulating NK cell activation and of the diversity of NK cell functions. In order to express their effector machinery, including cytotoxic granules and IFN- γ , NK cells need to be activated by cytokines and membrane interactions with DCs or monocytes/macrophages. **1) Activation.** These effector functions are then triggered upon recognition of target cells. This recognition occurs when the activation signals overcome the inhibitory signals received by NK cells as a result of the engagement of NK cell receptors. **2) Recognition.** Most NK cell inhibitory receptors recognize self-MHC class I molecules. Different NK cell activation receptors can recognize different types of ligands, including stress-induced self-molecules as illustrated by the NKG2D/MIC-A interaction, pathogen-derived molecules as illustrated by the interaction between NKp46 and the hemagglutinin of influenza virus, or MHC class I molecules associated with microbial components. **3) Functions.** NK cells can exert a variety of functions. These include direct microbicidal activities, either through the killing of infected cells or through noncytolytic control of pathogen replication, as well as a variety of immunoregulatory activities, such as the promotion of Th1 responses through the production of IFN- γ and the induction of DC maturation or the prevention of hemophagocytic lymphohistiocytosis through the killing of

activated macrophages. NK cell activity does not always benefit the host but can sometimes contribute to immunopathology. **(B)** Schematic representation of potential therapeutic strategies to harness NK cell activity for defense against infective agents. The promotion of **i)** NK cell activation and **ii)** NK cell-specific recognition of infected cells are two basic requirements for the successful design of NK cell-based immunotherapies against infectious agents. Different strategies can be used to meet these requirements, such as direct injection of cytokines versus activation of accessory cells by TLR stimulation for NK cell activation, and blockade of NK cell inhibitory receptors or promotion of the engagement of adequate NK cell activation receptors for target cell recognition. **(iii)** In certain diseases, such as HIV-1 infection, NK cells may exert detrimental functions that need to be dampened. **iv)** Finally, it should be possible to harness the specificity of NK cell receptors to redirect CD8 T cells or activated macrophages against the pathogen, through the engineering of adequate chimeric molecules (see section entitled 'Redirecting other immune cell specificity through ectopic transfer of NK cell recognition specificity for infectious agents' for details). aKIR: Activation KIR; aLy49: Activation Ly49 receptors; BM: Bone marrow; DC: Dendritic cell; FasL: Fas ligand; IFN: Interferon; iKIR: Inhibitory KIR; iLy49: Inhibitory Ly49 receptors; LFA: Lymphocyte function-associated antigen; LT: Lymphotoxin; Mφ: Macrophage; NK: Natural killer; NKp44L: NKp44 ligand; pMHC: MHC class I molecule loaded with a peptide; TLR: Toll-like receptor; TRAIL: Tumor necrosis factor-related apoptosis-inducing ligand; TRAIL-R: TRAIL receptor; ULBP1: UL16 binding protein 1.

Table 1
Natural killer cell implication in the physiopathology of exemplary human or mouse infections.

Pathogen	Host	Observation				
		Molecular mode of pathogen/infected cell recognition by NK cells	Ref.	Molecular mode of NK cell activation	Ref.	Escape of pathogen
<i>Virus</i> MCMV	Mouse	Engagement of NK cell activation receptors by viral or modified host ligands;		Cross-talk with DCs	[141,142]	Prevention of NKG2D ligand expression on infected cells
		-Viral m157/NKcell Ly49H	[79,80]	Direct stimulation by innate cytokines	[51]	Selection of escape mutations in m157
		-H2-K ^k presumably loaded with a viral peptide/NK cell Ly49P	[75]			Engagement of NK cell inhibitory receptors by viral or host ligands: -Ly49I/viral m157 -viral m144 mimicks host MHC class I
		-Other	[137]			
HIV-1	Human	Correlation of resistance to disease progression and KIR/MHC haplotypes	[42,43]			Alterations in NK cell subsets and functions
		Involvement of KIR3DS1 in recognition of infected cells	[36,47,48]			Modulation of the cytokine milieu
					[21,133]	Contribution to immunopathology through NKp44-dependent killing of uninfected CD4 T cells
					[147]	Correlation between NK cell and CD8 T-cell activity
					[148]	

Pathogen	Host	Observation					Ref.	Immunoregulatory functions	Ref.
		Molecular mode of pathogen/infected cell recognition by NK cells	Ref.	Molecular mode of NK cell activation	Ref.	Escape of pathogen			
Influenza	Human & mouse	Engagement of NKp44 and NKp46 by viral hemagglutinin	[84,138,139]	Cross-talk with DCs	[104,139]	Increase binding of inhibitory receptors KIR2DL1 and LIR1 on infected cells	[149,150]		
		NKp46 is involved in mouse resistance to influenza infection	[86]	Direct stimulation by innate cytokines	[60]	Modulation of the cytokine milieu	[150]		
		NKG2D-ULBP interaction	[139]						
Vaccinia	Human & mouse	Involvement of NKp30, NKp44 and NKp46 in recognition of infected cells	[88]	Mouse resistance to infection requires direct activation of NK cells by IFN- α/β	[54]	Direct infection of NK cells	[151]		
		Decreased NKG2A-mediated inhibition due to HLA-E decrease on infected cells	[140]			Modulation of the cytokine milieu	[152]		
<i>Parasite</i>						Inhibition of IFN γ receptor pathway	[153]		
Plasmodium	Human & mouse	Controversial role of pTEMPI in the regulation of NK cell functions	[156-158]	Direct stimulation by innate cytokines	[58,159]				
		ICAM-1-LFA1	[157]	Cross-talk with monocytes	[58,62]				
		FasL-Fas	[94]						
		Other	[40]						
Leishmania	Mouse	Direct engagement of TLR2 on NK cells by Leishmania lipophosphoglycan	[10]	Cross-talk with cDCs	[61]				
<i>Bacteria</i>									
Mycobacteria	Mouse	Engagement of NKG2D by ULBP1	[160,161]	Cross-talk with DCs	[162]			Regulation of CD8 T-cell functions	[163]