NK CELLS IN HIV INFECTION: PARADIGM FOR PROTECTION OR TARGETS FOR AMBUSH

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Abstract | Natural killer cells are a crucial component of the innate immune response to certain tumours and to various viruses, fungi, parasites and bacteria. HIV has infected more than 60 million people worldwide and has led to more than 23 million deaths. At present, there are ~40 million people who are living with HIV infection, and there were 5 million new infections in 2004. As part of the innate immune system, natural killer cells might have an important role in host defence against HIV infection, as well as in the control of HIV replication *in vivo*. In this regard, it is important to understand how natural killer cells and HIV interact. This Review focuses on the role of natural killer cells in controlling HIV infection and on the impact of HIV and HIV-viraemia-induced immune activation on natural-killer-cell function.

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Natural killer (NK) cells are a subset of lymphoid cells that function as important mediators of the innate immune defence against viruses and tumour cells[1]. They constitute ~15% of peripheral-blood lymphocytes and are also found in the liver, peritoneum and placenta[1]. NK cells were discovered because of their ability to spontaneously kill tumour-cell targets *in vitro*, which is the reason that they were named 'natural killer' cells[2] (FIG. 1). Early animal studies showed that RMA T-cell lymphomas grow progressively in syngeneic mice but that an MHC-class-I-deficient variant, RMA-S, is rejected by host NK cells *in vivo*[3]. These observations led to the identification of inhibitory NK-cell receptors (iNKRs), which recognize MHC class I molecules and block the cytotoxic function of NK cells[4,5] (BOXES 1,2). Activation of NK cells most probably results from the engagement of several receptors at the cell surface of NK cells — including cytokine receptors, adhesion molecules and cytotoxic activating NK-cell receptors — by their respective ligands at the cell surface of tumour cells and/or virus-infected cells[6,7]. The balance between stimulation through iNKRs and activating NK-cell receptors is fundamental to the regulation of NK-cell cytotoxic activity[6–8].

NK cells also mediate non-cytolytic suppression of viral replication. This occurs through the secretion of several chemokines, such as CC-chemokine ligand 3 (CCL3; also known as MIP1α), CCL4 (also known as MIP1β) and CCL5 (also known as RANTES), and several cytokines, such as interferon-γ (IFN-γ), tumour-necrosis factor (TNF) and granulocyte/macrophage colony-stimulating factor (GM-CSF)[1,9–11] (FIG. 2). Such soluble factors have an important role in inflammatory reactions, haematopoiesis, immune responses and activation of effector cells.

Two distinct subsets of NK cells have been described, and they are characterized by their relative expression of the cell-surface markers CD16 (also known as FcγRIII) and CD56 (REFS 12–14). The $CD16^{low/-}CD56^{hi}$ subset constitutes less than 10% of peripheral-blood NK cells and is important for production of chemokines and other cytokines. Cells of this subset express the high-affinity interleukin-2 (IL-2) receptor (which contains the α-, β- and γ-subunit) and produce large amounts of IFN-γ. However, these cells have poor cytotoxic activity against tumour-cell targets, indicating that they might regulate other cell types. By contrast, the $CD16^{hi}CD56^{low}$ subset, which produces

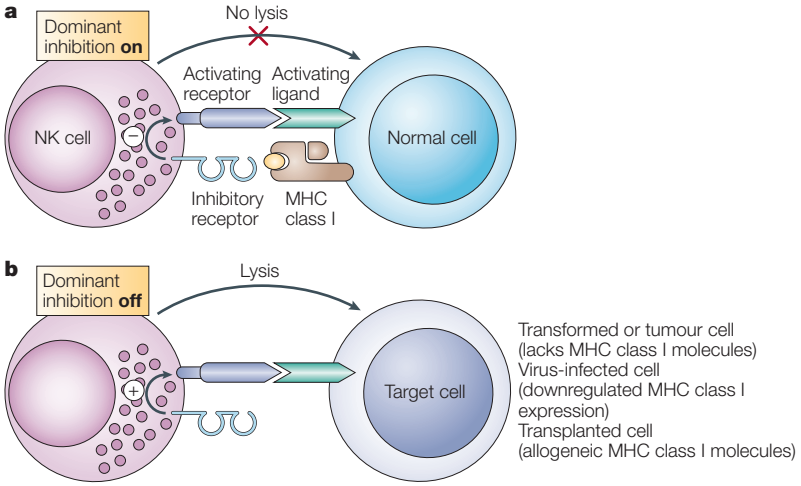

Figure 1 | **Natural-killer-cell recognition of target cells.** Natural killer (NK)-cell recognition of target cells is influenced by the level of expression of MHC class I molecules at the surface of the target cell. **a** | In the presence of MHC class I molecules on another cell, inhibitory NK-cell receptors (iNKRs) are triggered, leading to the delivery of inhibitory signals and, consequently, to lack of target-cell lysis. **b** | In the case of tumour cells or virus-infected cells, the cell-surface expression of MHC class I molecules is downregulated, so iNKRs are not triggered. Instead, positive signals through activating receptors dominate, which induces lysis of target cells.

little IFN-γ, constitutes 90% of peripheral-blood NK cells and is mainly responsible for natural cytotoxicity and ANTIBODY-DEPENDENT CELL-MEDIATED CYTOTOXICITY (ADCC)[12–14]. Furthermore, members of the killer-cell immunoglobulin-like receptor (KIR) family of iNKRs are preferentially expressed at the surface of cells of the $CD16^{hi}CD56^{low}$ NK-cell subset[12–14].

Owing to their ability to secrete various cytokines that promote differentiation of $CD4^+$ T cells and initiate effector responses of adaptive immune cells[15–17], NK cells are a crucial link between innate and adaptive immune responses[11,18,19], and they have an important role in the regulation of haematopoiesis[1]. Recently, it has been shown that NK cells interact with dendritic cells (DCs) and engage in an active crosstalk[11,18–20]. As a result, DCs prime NK cells, and the activated NK cells, in turn, secrete cytokines that can induce DC maturation, thereby promoting adaptive immune responses[11,19].

Given these functional capabilities, NK cells probably have a considerable role in the prevention and control of HIV infection. NK cells can eliminate HIV-infected target cells by direct lysis, by ADCC and by facilitating the priming of adaptive immune responses to HIV through their interactions with mature DCs[21–24]. But, despite the potential opportunity for NK-cell-mediated control of HIV, NK-cell responses that occur in individuals who are infected with HIV are dysfunctional.

NK-cell dysfunction in patients with AIDS was first described in the early 1980s[25,26], and several subsequent studies consistently described that defective NK-cell lysis was associated with progression of disease[21,27–29]. The introduction of ANTIRETROVIRAL THERAPY (ART) in the mid-1990s enabled physicians to control HIV replication in patients, reducing HIV viraemia significantly, to below the levels of detection *in vivo*. The use of ART led to a remarkable decrease in the occurrence of opportunistic infections, and it reduced the morbidity and mortality of individuals infected with HIV[30]. The use of ART also enabled scientists to study the effects of HIV viraemia on different aspects of immune defence, including NK cells by comparing NK-cell function in patients with and without detectable viraemia (that is, in the absence and presence of ART). Recently, there have been several important advances in our understanding of the expression and function of various NK-cell receptors in HIV infection. This Review focuses on the studies that have explored the role of NK cells in controlling HIV replication and/or the effects of HIV viraemia on NK cells.

Effect of NK cells on HIV infection

NK cells can contribute to the host immune response to HIV infection through cytolytic and non-cytolytic mechanisms.

Lysis of HIV-infected cells by NK cells. HIV is known to downregulate MHC class I expression at the surface of infected cells *in vitro*, thereby allowing it to escape recognition and lysis by $CD8^+$ cytotoxic T cells. These HIV-infected cells with low levels of MHC class I expression should then be susceptible to NK-cell-mediated lysis. Consistent with this, previous studies have shown that NK cells can lyse, either directly or through ADCC, tumour cell lines infected with HIV[31–33]. However, recent studies have addressed the role of MHC class I expression using autologous T-cell blasts infected with HIV as targets for NK cells *in vitro*[22,23,34]. These HIV-infected target cells have markedly downregulated their expression of MHC class I molecules but, surprisingly, are resistant to lysis by NK cells. This might be explained by the finding that HIV seems to selectively downregulate cell-surface expression of HLA-A and HLA-B molecules while preserving the expression of HLA-C and HLA-E molecules, thereby evading both $CD8^+$ T-cell responses and NK-cell-mediated lysis[34,35]. This observation is probably limited to those NK cells that lack HLA-C- and HLA-E-specific iNKRs and those target cells that specifically downregulate HLA-A and HLA-B molecules[23]. In this regard, HIV-viraemic states have been shown to be associated with an increase in the NK-cell subset expressing HLA-C-specific iNKRs, thereby increasing the proportion of NK cells that cannot kill HIV-infected $CD4^+$ T cells[36,37].

Suppression of HIV entry by NK cells. The CC-chemokines CCL3, CCL4 and CCL5, which are ligands for CC-chemokine receptor 5 (CCR5), can block entry of R5 VIRUSES to target cells by competitive inhibition of receptor binding[38–40]. Accordingly, NK cells, which produce large amounts of CCL3, CCL4 and CCL5, have been shown to suppress HIV replication *in vitro* by inhibiting CCR5-dependent entry of HIV to target cells[41–44]. In fact, CC-chemokines are the main soluble factors that are involved in NK-cell-mediated suppression of HIV replication. This differs from $CD8^+$ T cells, which suppress HIV replication by as-yet-unidentified non-chemokine factors[43].

ANTIBODY-DEPENDENT CELL-MEDIATED CYTOTOXICITY (ADCC). A cytotoxic mechanism by which an antibody-coated target cell is directly killed by a leukocyte that expresses Fc receptors, such as a natural killer (NK) cell, macrophage or neutrophil. A specific receptor for the constant region of IgG, FcγRIII (also known as CD16), is expressed at the surface of most NK cells and mediates ADCC.

ANTIRETROVIRAL THERAPY (ART). Combination treatment regimens of antiretroviral drugs that effectively suppress HIV replication and delay progression to AIDS. In general, ART includes three or more drugs, such as two nucleoside reverse-transcriptase inhibitors (NRTIs), one protease inhibitor and/or one non-NRTI. One new class of antiretroviral agent is a fusion inhibitor (enfuvirtide), which blocks the entry of HIV to cells, and it is generally used to treat individuals who are infected with multidrug-resistant HIV.

R5 VIRUS
An HIV strain that uses CC-chemokine receptor 5 (CCR5) as the co-receptor to gain entry to target cells.

X4 VIRUS
An HIV strain that uses CXC-receptor 4 (CXCR4) as the co-receptor to gain entry to target cells.

MOUSE CYTOMEGALOVIRUS
(MCMV). Immune responses to this herpesvirus-family member can suppress viral replication but do not completely eliminate the virus, leading to persistent infection. MCMV is highly homologous to human cytomegalovirus and is therefore often used as an *in vivo* model of chronic viral infection in a natural host.

SEROCONVERSION
The development of antibodies specific for HIV antigens. When individuals develop antibodies specific for HIV, they seroconvert from an antibody-negative state to an antibody-positive state. After infection with HIV, it might take from as little as 1 week to several months (or more) for antibodies to the virus to develop.

However, similar to $CD8^+$ T cells, NK cells can suppress endogenous replication of HIV by cell–cell contact, as well as through the production of soluble factors (FIG. 2). Although suppression of HIV replication by NK-cell-derived soluble factors is mainly restricted to R5 viruses, cell–cell-contact-mediated suppression affects both R5 and X4 VIRUSES[43]. The exact mechanism of cell–cell-contact-mediated suppression by NK cells has not yet been determined[42–44], and further studies are required to delineate the precise mechanisms of NK-cell suppression of HIV replication *in vivo*.

***Role of NK cells in resistance to HIV infection* in vivo.** Because NK cells can lyse targets by direct cytotoxicity before the recruitment of the adaptive immune response, they could have an important role in protecting a host from initially acquiring HIV infection. Recently, the NK-cell functions of Vietnamese intravenous drug users who seem to remain HIV seronegative despite several years of high-risk exposure to HIV were compared with those of HIV-infected patients and uninfected, control individuals[45]. NK cells from the exposed, uninfected individuals had higher cytolytic activity against various target cell lines than did NK cells from individuals who were either HIV-infected or HIV seronegative. Moreover, NK cells from the exposed, uninfected individuals produced considerably more of the chemokines CCL3, CCL4 and CCL5 and of the cytokines IFN-γ and TNF than did NK cells from HIV-positive individuals and HIV-negative volunteers[45]. It is probable that the increased NK-cell activity seen in these exposed, uninfected individuals reflects an activated immune system as a result of exposure to multiple systemic infections[46]. Consistent with this, exposure to various viruses, such as MOUSE CYTOMEGALOVIRUS, can lead to activation of mouse NK cells[47]. However, NK-cell activity (both cytolytic and secretory) was lower both before and after SEROCONVERSION among intravenous drug users who seroconverted during the study than among individuals who remained uninfected for the duration of the study[45]. Therefore, it seems that the increased NK-cell activity of exposed, uninfected Vietnamese intravenous drug users is associated with resistance to acquiring HIV infection. These findings support the hypothesis that NK cells contribute to protection from HIV infection in certain individuals[45]. However, it is unclear whether this increased NK-cell activity that is seen among exposed, uninfected individuals is associated with a genetic background that favours NK-cell activation by the expression of certain NK-cell receptors (either activating or inhibitory) and/or their ligands[48] or whether it is associated with environmental factors.

Two recent studies have described the influence of genotype on the control of HIV viraemia and disease progression[48,49]. One study showed that the expression of HLA-Bw4, a ligand for a KIR expressed at the surface of NK cells, was associated with control of HIV viraemia and slower progression to AIDS[49]. Another recent study reported that the presence of the activating KIR allele KIR3DS1 and the HLA-B allele HLA-Bw4 Ile80 are associated with delayed progression of HIV infection to AIDS[48]. In the absence of the KIR3DS1 allele, HLA-Bw4 Ile80 expression did not protect against disease progression[48]. Furthermore, presence of the KIR3DS1 allele in the absence of the HLA-Bw4 Ile80 allele was associated with rapid progression to AIDS among HIV-infected individuals, indicating that there is an EPISTATIC ASSOCIATION between these two loci[48]. These genetic studies, together with the functional studies of exposed, uninfected individuals, indicate a protective role for NK cells in the early stages of HIV infection[45,48,49].

Effect of HIV viraemia on NK cells

HIV viraemia induces several phenotypic and functional abnormalities in NK cells[36,43,50–53] (FIG. 3; TABLE 1). HIV might exert these effects on NK cells in three ways: direct infection of NK cells, direct binding of HIV to chemokine receptors at the surface of NK cells and indirect effects on NK cells as a result of generalized HIV-induced immune activation.

First, for direct infection by HIV, cells must express CD4, but most freshly isolated NK cells lack cell-surface expression of CD4 (REF. 36). They do, however, express the chemokine co-receptors for HIV, CCR5 and CXC-chemokine receptor 4 (CXCR4)[50], but these receptors alone are unlikely to allow productive infection of NK cells by HIV *in vivo*[36]. However, a recent

Box 1 | Natural-killer-cell receptors

Natural killer (NK) cells express two types of cell-surface receptor, inhibitory and activating[4–6]. Of these, inhibitory NK-cell receptors (iNKRs) are highly specific for MHC class I molecules and comprise two families: the killer-cell immunoglobulin-like receptor (KIR) family and the C-type-lectin family of receptors[4,5,90,91]. Despite their disparities in molecular structure, all iNKRs have intracytoplasmic domains known as immunoreceptor tyrosine-based inhibitory motifs (ITIMs), which mediate recruitment and activation of the intracellular tyrosine phosphatases SHP1 (SRC-homology-2-domain-containing protein tyrosine phosphatase 1) and SHP2 (REF. 5). Interaction between iNKRs and MHC class I molecules is the dominant check-point in the inhibition of NK-cell cytotoxicity and other effector functions[4,5]. Several KIRs (such as KIR2DS1 and KIR2DS2) have typical HLA-class-I-specific extracellular domains but are activating in function as a consequence of the absence of ITIMs in their intracytoplasmic domains[4,5].

Activating receptors, such as the recently described natural cytotoxicity receptors (NCRs) NKp30 (NK-cell protein 30), NKp44 and NKp46 (REF. 92), provide the 'on signal' for stimulation of NK cells during their interaction with target cells[6]. These receptors are readily triggered by ligands expressed at the surface of target cells that lack MHC class I molecules, and they have an important role in the killing of several NK-cell-susceptible tumour targets, as shown by experiments in which activating receptors are blocked with specific antibodies[6]. NKG2D (NK group 2, member D) is another activating receptor, and it binds the endogenous ligands ULBP (cytomegalovirus UL16-binding protein), MICA (MHC-class-I-polypeptide-related sequence A) and MICB, and mediates lysis of target cells[9,78].

NK cells have several accessory receptors that regulate their behaviour in response to various targets that lack MHC class I molecules. These include NKp80, NKR-P1A (NK-cell receptor protein 1A; also known as CD161), CD96 and 2B4 (also known as CD244)[6,7]. Engagement of these receptors can increase cytotoxic responses and cytokine production that have been induced by engagement of other receptors, so these accessory receptors are known as co-activating or co-stimulatory molecules[6]. It has been proposed that simultaneous engagement of these receptors and other activating receptors might override the effect of iNKR engagement and initiate NK-cell effector functions[6,7].

EPISTATIC ASSOCIATION
In genetic epidemiology, an epistatic effect is the modification of the risk that is conferred by one marker by the presence of a marker from an unrelated gene (that is, an unlinked gene–gene interaction).

study identified a subset of NK cells that do express CD4, together with CCR5 and CXCR4, and these cells could be productively infected *in vitro* with HIV in a CD4-dependent manner[54]. Moreover, longitudinal analysis of viral DNA concentrations in NK cells from patients who are receiving ART indicated that NK cells can be persistently infected and could therefore be a reservoir of HIV[54]. This might therefore contribute to the NK-cell dysfunction that is observed in individuals who are infected with HIV.

Second, as previously mentioned, NK cells express both chemokine co-receptors of HIV, through which HIV might trigger intracellular signalling that could result in dysregulated NK-cell activation or trafficking. In T cells, although HIV envelope proteins can bind and trigger chemokine-receptor signalling, simultaneous ligation of CD4 is essential for the induction of apoptosis[55,56]. Interestingly, the expression of CCR5, but not CXCR4, at the surface of NK cells from HIV-viraemic patients is markedly higher than that observed for HIV-seronegative individuals[50], which might facilitate interaction between HIV and NK cells (although this remains to be proven).

Third, HIV infection can induce inappropriate NK-cell activation through indirect effects that are a consequence of ongoing HIV replication and the associated immune response. NK cells from HIV-viraemic individuals and from patients with disorders that are associated with aberrant immune activation (such as Wegener's granulomatosis) have increased CCR5 expression, indicating that CCR5 expression is influenced by immune-activation states[50]. Given the complexity of these interactions, it is often difficult to determine the relative contributions that the direct effects of HIV and the effects of HIV-associated immune activation have on altered NK-cell phenotype and function.

Dichotomous expression of iNKRs and natural cytotoxicity receptors. The number of NK cells that express iNKRs has been found to be increased in HIV-viraemic individuals, and this could account for an increased receptor-specific inhibition of NK-cell cytolytic function *in vitro*[36,50,57–59]. That, in HIV infection, the expansion of NK cells expressing iNKRs has an important functional relevance is supported by the following observation: despite the selective decrease in MHC class I expression that generally occurs in HIV infection *in vitro*[35], NK cells from HIV-infected patients cannot lyse infected autologous T-cell blasts[22], unless the interaction between specific HLA haplotypes and the corresponding iNKRs is blocked[23]. Although several studies have described this proliferation of NK-cell populations expressing various iNKRs — including CD94 (also known as KLRD1), leukocyte immunoglobulin-like receptor 1 (LIR1; also known as ILT2 and CD85j) and various KIR-family members — in viraemic patients[36,50,58–61], increased expression of individual inhibitory KIRs in HIV-infected individuals has not been consistently reported, with some studies describing no change (TABLE 1). These discrepancies probably result from differences in several factors: the disease states of the individuals with HIV viraemia, the types of sample that were used (that is, whole blood versus purified NK cells) and the methods that were used to analyse receptor expression. However, all of these studies have consistently shown that the number of NK cells that express iNKRs is normalized with control of HIV viraemia by ART, underscoring the effect of HIV viraemia on the abnormal expression of iNKRs. Collectively, these studies imply that NK cells in HIV-infected individuals have impaired cytolytic activity as a result of increased iNKR expression, thereby leading to their inability to control HIV replication *in vivo*.

If the dominant inhibitory effects of iNKRs on NK-cell killing of MHC-class-I-expressing target cells are overcome, as would be expected by the virus-induced downregulation of HLA class I expression, then the triggering of activating NK-cell receptors by their ligands should induce NK-cell cytolytic activity. Analysis of expression of activating receptors at the surface of NK cells from HIV-viraemic individuals has shown that all three natural cytotoxicity receptors (NCRs) — NK-cell protein 30 (NKp30), NKp44 and NKp46 — are expressed at markedly lower levels than by the NK cells of HIV-negative individuals[36,62]. Importantly, control of viraemia by ART (for more than two years, in this study) has been shown to be associated with normalization of expression of the NCRs[36]. Therefore, despite the persistent and aberrant activation of NK cells as a consequence of their chronic exposure to HIV[63], NK cells have an NCR^{low} phenotype, which

Box 2 | Natural-killer-cell recognition of target cells

Natural killer (NK) cells do not use an antigen-recognition receptor for distinguishing between self, non-self and altered self, unlike T and B cells[14]. They also do not use pathogen-recognition receptors, such as those used by monocytes and macrophages[7]. Instead, NK-cell responses depend on the outcome of the integration of intracellular signals that are transmitted from inhibitory and activating receptors. The recognition of targets by NK cells involves three main mechanisms.

The first mechanism is known as missing-self recognition, which refers to inhibitory-receptor recognition of self-proteins that are expressed by normal, healthy cells but that are altered or absent from the surface of infected cells and tumour cells[78]. The dominant signal that is transmitted from inhibitory NK-cell receptors (iNKRs) contributes a fail-safe mechanism for avoiding the induction of a response to self (such as to MHC class I molecules) and therefore inadvertent activation of NK cells. When both iNKRs and activating NK-cell receptors are triggered, the net result is determined by the balance of the strength of the signals that are transmitted by these receptors[6,7].

The second mechanism is known as induced self-recognition, and this involves the receptor NKG2D (NK group 2, member D)[93] recognizing self-proteins — such as ULBP (cytomegalovirus UL16-binding protein), MICA (MHC-class-I-polypeptide-related sequence A) and MICB — that are upregulated by infected cells or tumour cells[6,7,78,93].

The third mechanism involves recognition of pathogen-encoded molecules, and this mechanism is exemplified by interactions of the mouse receptor Ly49H. In general, Ly49 molecules bind MHC class I molecules, have immunoreceptor tyrosine-based inhibitory motifs (ITIMs) in their cytoplasmic tails[94] and are inhibitory in function. Ly49H contains immunoreceptor tyrosine-based activation motifs (ITAMs) instead of ITIMs in its cytoplasmic tail and is therefore activating in function[94]. Ly49H binds m157, an MHC-like protein that is encoded by mouse cytomegalovirus[47,95,96], indicating that Ly49H can function as a pathogen-specific receptor that leads to NK-cell activation. In humans, such recognition by NK cells might be mediated by the receptors NKp44 (NK-cell protein 44) and NKp46, which bind the influenza-virus encoded protein haemagglutinin[97].

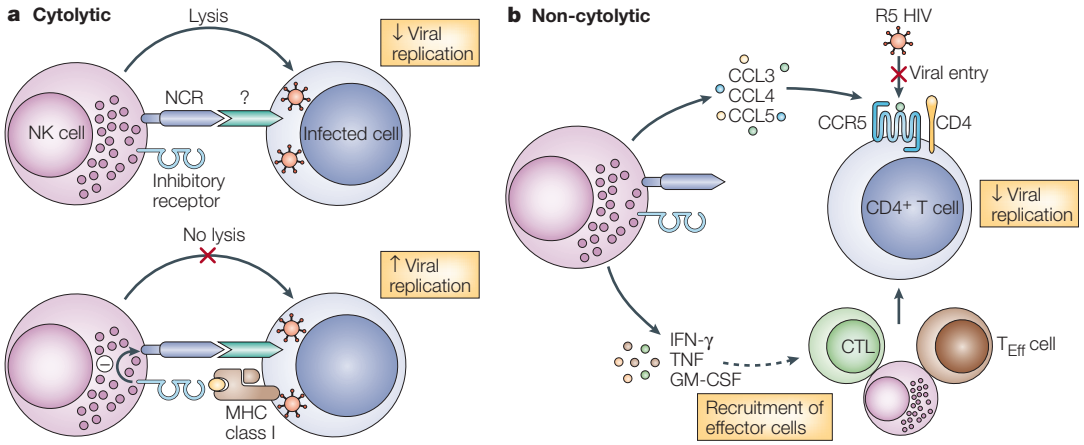

Figure 2 | **Cytolytic and non-cytolytic mechanisms that are mediated by natural killer cells for the control of HIV infection. a** | Natural killer (NK) cells recognize virus-infected target cells that lack MHC class I expression at their surface, and become activated. Activation of NK cells leads to lysis of target cells, which is mediated by perforin and granzymes, and therefore to the control of viral replication. However, infection with certain viruses (such as HIV) might not lead to downregulation of expression of all MHC class I molecules; in this case, NK cells are not activated and cannot lyse target cells, leading to uncontrolled viral replication. **b** | NK cells can also inhibit HIV replication by secreting the chemokines CC-chemokine ligand 3 (CCL3), CCL4 and CCL5, which inhibit entry of HIV to $CD4^+$ T cells. NK cells also secrete several other cytokines, including interferon-γ (IFN-γ), tumour-necrosis factor (TNF) and granulocyte/macrophage colony-stimulating factor (GM-CSF), that might inhibit HIV replication by recruiting other effector cells *in vivo*. CCR5, CC-chemokine receptor 5; CTL, cytotoxic T lymphocyte; NCR, natural cytotoxicity receptor; R5 HIV, CCR5-tropic HIV; T_{Eff} cell, effector T cell.

probably affects NK-cell-mediated clearance of virus-infected cells, of tumour cells and of opportunistic infections that normally occur in the late stages of HIV infection (FIG. 3).

By contrast, the expression and function of another important activating NK-cell receptor, NKG2D (NK group 2, member D), and several activating co-receptors, including 2B4, NKp80 and NTBA (NK-, T- and B-cell antigen), are maintained in HIV-viraemic individuals[36,50,58,59], perhaps explaining the residual NK-cell cytolytic activity that has been observed in such patients (TABLE 1).

As previously mentioned, the presence of activating forms of KIRs (such as KIR3DS1) at the surface of NK cells is associated with delayed progression of HIV infection, which highlights the importance of activating KIRs in the pathogenesis of AIDS[48]. Because the antibodies that are available at present cannot be used to separate inhibitory and activating KIR-expressing cells (because the extracellular domains of inhibitory and activating KIRs are similar), phenotypic studies must be accompanied by functional studies. In this regard, such studies have confirmed that it is the inhibitory KIRs that are overexpressed[64]. Further studies that specifically address the function of activating KIRs are warranted, to gain an understanding of their protective role in the pathogenesis of AIDS.

Proliferation of defective $CD16^{hi}CD56^-$ NK cells. Several changes in the cell-surface expression of various NK-cell receptors are seen in HIV-infected individuals (TABLE 1). Phenotypic analysis of NK cells from HIV-infected individuals has shown that there is a selective loss of the cytolytic, $CD16^{hi}CD56^{low}$ NK-cell subset and an expansion of the $CD16^{hi}CD56^-$ subset[36,51–53,65]. The decreased expression of CD56 at the surface of NK cells from HIV-infected individuals is only partially restored by suppression of HIV replication *in vivo* by ART[36,66]. In untreated individuals, the $CD16^{hi}CD56^-$ subset expresses higher levels of iNKRs and markedly lower levels of NCRs, and secretes lower amounts of cytokines, than the $CD16^{hi}CD56^{low}$subset; in addition, it is defective at lysis of targets[64]. These data indicate that the proliferation of a highly dysfunctional $CD16^{hi}CD56^-$ NK-cell population in HIV-viraemic individuals is at least partially responsible for the observed defects in NK-cell function[64,67].

Defective non-cytolytic suppression of HIV. As previously mentioned, NK cells can mediate potent suppression of endogenous HIV replication *in vitro* by cell–cell contact and by the secretion of soluble factors, and this suppression is comparable to that mediated by $CD8^+$ T cells[43]. However, the degree of suppression by NK cells was shown to be more marked in patients who did not have detectable HIV viraemia than in patients with detectable viraemia[43]. In addition, there was an inverse correlation between the level of plasma viraemia at the time of study and the ability of NK cells to suppress endogenous HIV replication[43]. Moreover, the suppression of HIV replication by soluble factors that are secreted by NK cells was mediated almost entirely by CC-chemokines, and the ability of NK cells to secrete CC-chemokines was markedly lower in patients with viraemia than in those without viraemia[43]. Collectively, these findings indicate that HIV-induced inhibition of NK-cell function involves mechanisms that lead to diminished secretion of CC-chemokines[43].

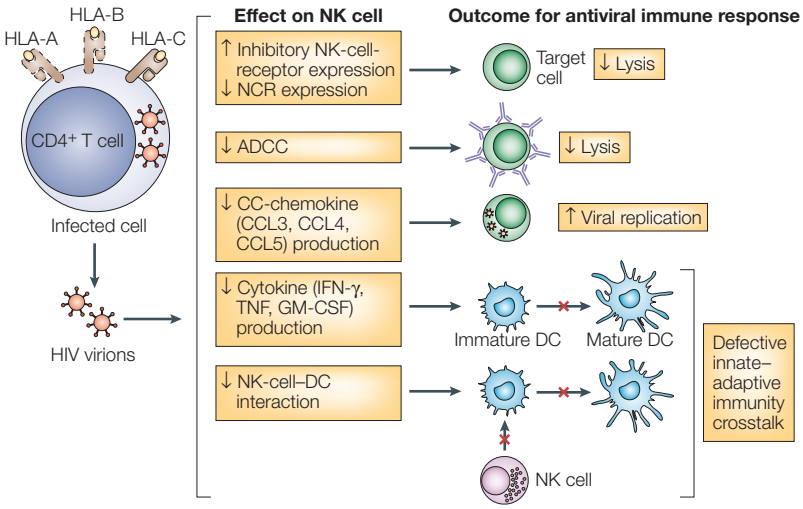

Figure 3 | **Effect of HIV viraemia on natural-killer-cell function.** HIV viraemia affects natural killer (NK)-cell phenotype and function in several ways, which consequently influences the interactions of NK cells and other immune effector cells that are important for the initiation and maintenance of immune responses to HIV. HIV viraemia reduces the expression of various activating receptors (natural cytotoxicity receptors, NCRs) at the surface of NK cells while increasing the expression of inhibitory NK-cell receptors. The net result of this dichotomous effect on NK-cell-receptor expression is a reduced ability to lyse virus-infected target cells. HIV viraemia also inhibits the secretion of CC-chemokines by NK cells, which reduces the ability of NK cells to suppress HIV entry by blocking CC-chemokine-receptor binding of HIV. HIV viraemia also leads to reduced secretion of pro-inflammatory cytokines — such as interferon-γ (IFN-γ) and tumour-necrosis factor (TNF) — and granulocyte/macrophage colony-stimulating factor (GM-CSF) by NK cells. This impairs the interaction of NK cells with other cellular components of the adaptive immune system, including dendritic cells (DCs). HIV-envelope-specific antibody-dependent cell-mediated cytotoxicity (ADCC) is impaired in HIV-infected individuals at later stages of disease. CCL, CC-chemokine ligand.

Defective ADCC in HIV infection. Over the past two decades, several studies have described impairments in NK-cell cytolytic activity during HIV infection, including in ADCC (which is mediated by CD16) and in direct cytolytic activity against HIV-infected tumour cells or against targets that have been transfected with HIV envelope proteins *in vitro*[21,25,26,28,29,61,68–70]. With regard to ADCC, impairments were seen is some individuals in the early stages of HIV infection, indicating an early impairment of NK-cell function by HIV[28,29] (FIG. 3). Moreover, there was also a marked inverse correlation between the titres of HIV-envelope-specific ADCC and the stage of HIV infection[71], as well as between the degree of impairment of NK-cell cytotoxicity and the concentration of HIV virions in the plasma[72].

Defective response of NK cells to cytokines. Several studies have also shown that lack of cytokine responsiveness is a contributing factor to NK-cell dysfunction in HIV-infected individuals[36,70,73]. *In vitro* activation of NK cells from HIV-viraemic individuals, using recombinant IL-2, could not restore their capacity to lyse targets[36]. In addition, impaired cytotoxicity of recombinant-IL-2-activated NK cells is associated with a higher probability of progression to AIDS[70]. Several studies, however, have described the ability of cytokines, particularly IL-15, to augment the direct cytotoxic potential of NK cells against HIV-infected autologous peripheral-blood mononuclear cells[74] and to augment ADCC, using HIV gp120 (glycoprotein 120)-coated target cells[75]. These findings indicate a potential role for cytokine-based therapies to augment NK-cell function in HIV-infected individuals. Clinical trials would be needed to further pursue this concept.

Effect of ART on function of NK cells. Since the advent of ART, investigators have shown varying degrees of improvement in NK-cell function associated with control of HIV viraemia[36,37,50,58,59,72,76,77]; however, so far, there is no consensus on the degree to which adequate suppression of ongoing HIV replication by ART restores NK-cell function. Longitudinal studies that monitor comprehensive NK-cell function in HIV-infected patients are needed so that we can more precisely address the degree of restoration of NK-cell function that is associated with ART-induced suppression of HIV replication.

Interaction between NK cells and DCs. Interaction with DCs and priming of the adaptive immune response is another important function of NK cells[11,18,19,78]. Several studies have implicated a liaison between NK cells and DCs in regulation of the maturation and function of DCs, thereby linking the innate and adaptive immune responses[11,20,79]. In a recent study addressing the functional integrity of NK-cell–DC interactions in early and chronic stages of HIV infection[24], the ability of NK cells to kill autologous, immature, monocyte-derived DCs was only weak, and it was inversely proportional to the level of viraemia at early stages of infection and directly associated with the number of $CD4^+$ T cells[24]. The exact physiological role of such NK-cell-mediated lysis of immature DCs is not clearly understood. A recent study investigated the function and numbers of DCs in HIV-infected children who are receiving ART[77]. The authors reported that there were fewer plasmacytoid and myeloid DCs, as well as fewer mature NK cells, associated with HIV viraemia in patients who had declining $CD4^+$ T-cell numbers than in those who had a similar level of viraemia but stable $CD4^+$ T-cell numbers. These changes were largely reversed in children who received ART and who could suppress the viral load. However, there was selective loss of mature, $CD16^+CD56^+$ NK-cell receptor protein 1A (NKR-P1A)$^+$ NK cells and a suppression of the ability of plasmacytoid DCs to secrete IFN-α[77]. Similar findings have also been described for HIV-infected adults[72].

It has recently been proposed that $CD16^{hi}CD56^{low}$ NK cells might exert a quality control over DCs that are undergoing maturation, through an editing process that could positively select DCs that, after antigen uptake, express optimal levels of HLA class I molecules[11,18–20,78,79]. Moreover, it has been proposed that NK cells might directly promote the maturation of DCs at inflammatory sites. The result of this process could be the selection and/or promotion of the 'optimal' DCs, which could induce efficient priming of the T-helper-1-cell adaptive immune response after they have migrated to the lymph nodes[80]. Recently, the cellular mechanisms by which these two components of the innate

immune system interact have been delineated[81–83]. These studies have shown the significance of NK-cell membrane-bound receptors (in particular, NKG2A and NKp30) and of secretion of pro-inflammatory cytokines by NK cells (in particular, IFN-γ, TNF and GM-CSF) in their interactions with DCs[82,83]. Recent studies of NK-cell function in HIV-infected individuals have described decreased expression of NKG2A and NKp30, as well as decreased secretion of IFN-γ, TNF and GM-CSF, associated with the HIV-viraemic state[36,64], indicating that there might be abnormalities in the NK-cell–DC interactions during HIV infection (FIG. 3). The role of NK-cell–DC interactions in the pathogenesis of HIV infection is an area of active investigation.

Future directions

Recent advances in delineating the biology of NK cells, particularly the identification of novel receptors and their ligands, have improved our understanding of the potential role of NK cells in the immunopathogenesis of HIV infection[6,7,81]. However, it is still unclear whether NK cells control HIV replication *in vivo*. Experiments in which NK cells are depleted from simian immunodeficiency virus (SIV)-infected animals are required to determine the effect of NK cells on the level of SIV viraemia. However, in monkeys, NK cells have a different phenotype than that described for humans[84]. CD56, the most common marker that is used to identify human NK cells, is absent from simian NK cells, whereas it is present on simian monocytes[85].

Table 1 | **Effect of HIV viraemia on natural-killer-cell receptors**

Receptor	Function	Ligand specificity	Effect of HIV viraemia on NK-cell-receptor expression	Effect of ART on NK-cell-receptor expression	References
CD16 (FcγRIII)	ADCC	IgG	No change	No change	14,36,51–53, 60,64,65,98
CD56	None	CD56, NCAM	Decrease	Restoration to normal levels	14,36,51–53, 60,64,65,98
KIR2DL2 (CD158b)	Inhibitory	HLA-Cw2, HLA-Cw4, HLA-Cw5, HLA-Cw6	No change, or increase	Restoration to normal levels	4,5,7,36,50,58–60,64
KIR2DL1 (CD158a)	Inhibitory	HLA-Cw1, HLA-Cw3, HLA-Cw7, HLA-Cw8	No change, or increase	Restoration to normal levels	4,5,7,36,50,58–60,64
KIR3DL1 (CD158e1)	Inhibitory	HLA-Bw4	No change, or increase	Restoration to normal levels	4,5,7,36,58,60,64
KIR3DL2 (CD158k)	Inhibitory	HLA-A3, HLA-A11	No change	No change	4,5,7,36,64
LIR1 (ILT2, CD85j)	Inhibitory	HLA-A, HLA-B, HLA-C, HLA-G	No change, or increase	Restoration to normal levels	4,5,36,64
p75 (AIRM1)	Inhibitory	ND	No change	No change	64,99
CD94 (KLRD1)*	ND	ND	No change, or increase	Restoration to normal levels	4,5,7,36,50,58, 60,64
NKG2A (CD159a)	Inhibitory	HLA-E	Decrease	Restoration to normal levels	4,5,7,36,64
NKG2D	Activating	MICA, MICB, ULBPs	No change	No change	6,7,36,64
NKp46 (NCR1)	Activating	Influenza-virus haemagglutinin, others unknown?	No change	Restoration to normal levels	6,36,62,64
NKp30 (NCR3)	Activating	ND	Decrease	Restoration to normal levels	6,36,62,64
NKp44 (NCR2)	Activating	Influenza-virus haemagglutinin, others unknown?	Decrease	Restoration to normal levels	6,36,62,64
NKp80	Co-activating	ND	No change	No change	6,36,64
NTBA	Co-activating	NTBA	No change	No change	36,64,93
2B4 (CD244)	Co-activating	CD48	No change	No change	6,36,50,64
NKR-P1A (CD161)	ND	ND	No change	No change	7,50,58
CCR5	Chemokine receptor	CCL3, CCL4, CCL5	Increase	Restoration to normal levels	50
CCR4	Chemokine receptor	CCL3, CCL4, CCL5	No change	No change	50

*CD94 forms heterodimers with members of the natural killer group 2 (NKG2) family, including NKG2A and NKG2C. ADCC, antibody-dependent cell-mediated cytotoxicity; AIRM1, adhesion inhibitory receptor molecule 1; CCL, CC-chemokine ligand; CCR, CC-chemokine receptor; FcγRIII, low-affinity Fc receptor for IgG; ILT2, immunoglobulin-like transcript 2; KIR, killer-cell immunoglobulin-like receptor; KLRD1, killer-cell lectin-like receptor D1; LIR1, leukocyte immunoglobulin-like receptor 1; MIC, MHC-class-I-polypeptide-related sequence; NCAM, neural cell-adhesion molecule; NCR, natural cytotoxicity receptor; ND, not determined; NK, natural killer; NKp, NK-cell protein; NKR-P1A, NK-cell receptor protein 1A; NTBA, NK-, T- and B-cell antigen; p75, protein 75; ULBP, cytomegalovirus UL16-binding protein.

TOLL-LIKE RECEPTORS (TLRs). A family of receptors that are homologous to *Drosophila melanogaster* Toll. TLRs recognize conserved molecular patterns that are present in pathogens, such as lipopolysaccharide in the bacterial cell wall.

CPG-CONTAINING OLIGODEOXYNUCLEOTIDE (CpG ODN). An ODN that includes a cytosine residue joined by a 5′-to-3′ phosphodiester linkage to a guanine residue as part of a normal DNA strand. This sequence is highly immunogenic and induces an innate immune response through interaction with Toll-like receptor 9.

Moreover, in monkeys, several NK-cell markers are also expressed by $CD8^+$ T cells, and these markers will need to be avoided as targets in depletion experiments. A recent study has indicated that NKp80 and NKG2A can be used as markers to identify NK cells in rhesus macaques and pig-tailed macaques[86]. Further characterization of phenotype and function of NK cells in these animal models would be invaluable for future studies on the role of NK cells in the control of HIV viraemia.

NK cells can also be strategically targeted to HIV-infected cells using antibodies or antibody fragments[87]. A recent study has shown that NK cells can be successfully targeted to lyse HIV-infected target cells *in vitro* using a highly polymerized chimeric IgG1–IgA fusion protein that, because of its capacity to extensively crosslink CD16, activates NK cells to lyse HIV-infected target cells *in vitro*[87]. Such novel molecules that could potentially be developed to target NK cells and lyse HIV-infected cells *in vivo* are being investigated.

Although it is clearly established that HIV induces several alterations in NK-cell phenotype and function *in vitro* and *in vivo*, it is not clear precisely how HIV interacts with NK cells. Further research is warranted to investigate whether there are novel molecules at the surface of NK cells that could be targets for HIV envelope proteins.

Finally, understanding the role of NK cells in the development of mucosal immunity to HIV infection would be invaluable. At present, it is unclear whether NK cells are recruited to the vaginal and/or rectal mucosal surfaces and, if so, whether they render protection against acquiring HIV or SIV infection. NK cells express TOLL-LIKE RECEPTOR 9 and can be activated by CPG-CONTAINING OLIGODEOXYNUCLEOTIDES (CpG ODNs)[88]. A recent study using a mouse mucosal herpesvirus-infection model showed that protection against infection could be achieved through the recruitment of activated NK cells to the mucosal surfaces by immunization with CpG ODNs[89]. Similar experiments carried out using SIV-infected macaques should determine the definitive role of NK cells in protection against mucosal infection. If NK cells are proven to be effective, then development of microbicides that activate NK cells would be of value in preventing HIV transmission.

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The authors declare no competing financial interests.

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ERRATUM

NK CELLS IN HIV INFECTION: PARADIGM FOR PROTECTION OR TARGETS FOR AMBUSH

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When published, the thirteenth row in Table 1 contained incorrect information. The corrected row (together with the column headings) is shown below.

Receptor	Function	Ligand specificity	Effect of HIV viraemia on NK-cell-receptor expression	Effect of ART on NK-cell-receptor expression	Refs
NKp46 (NCR1)	Activating	Influenza-virus haemagglutinin, others unknown?	Decrease	Restoration to normal levels	6,36, 62,64