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REVIEW

# Natural killer cell heterogeneity: cellular dysfunction and significance in HIV-1 immuno-pathogenesis

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**Abstract** Natural killer (NK) cells are innate immune effectors that provide first line of defence against viruses. Human NK cells are heterogeneous in nature, and their functions rely on a dynamic balance between germ-line-encoded activating and inhibitory receptors. HIV-1 infection results in altered NK cell receptor repertoire and impaired effector functions including the ability to lyse virus-infected cells and secretion of antiviral cytokine IFN-γ. Over the last decade, additional NK cell subset-specific molecules have been identified, leading to emergence of a more complex cellular diversity than previously thought. Herein, we discuss NK cell subset redistribution, altered receptor repertoire and influence of interaction of polymorphic leucocyte antigen (HLA) and killer cell immunoglobulin-like receptors (KIR) on HIV-1 disease progression.

**Keywords** Innate immunity · CD56 dim and bright · Granzyme B · Antibody-dependent cellular cytotoxicity · Highly active antiretroviral therapy

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## Introduction

Metazoans have evolved intrinsic defence systems to confront invading microbes and ensure their elimination from the body. The first line of natural protection is provided by the innate immune system and later by pathogen-specific adaptive immunity. The innate immune responses against viral infections are largely provided by NK cells, representing typically 5–15 % of human peripheral blood lymphocytes [1–3]. Phenotypically, human NK cells are characterised as $CD3^-$ $CD56^+$ lymphocytes expressing CD16, the FcγRIIIA receptor. According to the newly adapted nomenclature of innate lymphoid cells (ILC), NK cells are placed into group 1 of ILC family, based on T-box transcription factor, EOMES and/or T-bet expression and interferon-gamma (IFN-γ) production [4]. Before activation, NK cells need interaction between inhibitory receptors and MHC class-1 molecules; otherwise, they remain hyporesponsive. This crucial step in NK cell development is called education and is accompanied by changes in the NK cell repertoire. However, the educational stage of an individual NK cell can be assessed only by functional means since markers have not been identified [5]. Unlike cytotoxic $CD8^+$ T lymphocytes (CTLs), NK cells selectively recognise and kill target cells lacking MHC class-1 expression. NK cell's direct or CD16-mediated antibody-dependent recognition of target cells triggers release of immune-regulatory cytokines, such as IFN-γ, tumour necrosis factor-alpha (TNF-α) and granulocyte–macrophage colony-stimulating factor (GM-CSF), and cytolytic factors including perforins (Pfr) and granzymes (Gr), which cause apoptosis via caspase-8/Bid or by death receptor pathways using Fas-FasL and TRAIL [6].

In addition to their presence in the peripheral blood, NK cells also exhibit diverse tissue distribution, referred to as

tissue-resident NK (trNK) cells [7]. For example, the uterine, mucosal and liver-resident NK cells phenotypically differ from their blood counterpart [8]. Peripheral blood NK cell diversity is highly complex; recent studies have described more than a thousand phenotypes [9] sharing NK cell receptors (NKRs), across the leucocyte lineages [10]. However, their significance in human pathophysiology is elusive. Major NKRs consist of natural cytotoxicity receptors (NCR-NKp30, NKp44, NKp46), CD16, the killer-immunoglobulin (Ig)-like receptors; inhibitory (iKIR) and activating (aKIR), and C-type lectin-like receptors (NKG2D, CD94/NKG2-A, -C). Binding of activating receptors including CD16 to their putative ligands triggers activating signal either via cytoplasmic immune-receptor tyrosine-based activating motifs (ITAM), ITAM-bearing adaptor molecules, such as DAP10 and DAP12, or phosphorylation of intracellular domains lacking any ITAMs as in the case of DNAM-1 [11]. In contrast, inhibitory receptors transduce their signals via immune-receptor tyrosine-based inhibitory motifs (ITIM) [12]. The balance of activating receptors including CD16 and inhibitory receptor signalling determines whether the cell will be activated and can thus lyse the target cell.

## HIV-1 infection and host immune responses

HIV-1 virus infection is responsible for causing acquired immunodeficiency syndrome (AIDS), manifested by massive $CD4^+$ T cell depletion associated with AIDS-defining illness. This includes multiple infections by viruses and bacteria including tuberculosis, and lymphomas. However, individuals with greater than 350 cells/μl are at lower risk of developing AIDS-defining diseases. A perturbed immune system is the hallmark of HIV-1/AIDS, in particular $CD4^+$ T cells which are preferentially targeted by HIV-1. Virus replication within $CD4^+$ T cells causes suppressed proliferation, IL-2 production and cell death, resulting in the absence of help provided to $CD8^+$ T cells, B cells and others cell types, including NK cells. During early infection, HIV-specific cytotoxic $CD8^+$ T lymphocytes (CTLs) are able to suppress virus replication but fail to contain for prolong period of time [13] with the exception of long-term non-progressors (LTNPs) and Elite controllers (ECs). It is believed that the prolonged infection may result in viral mutation enabling them to escape from CTL-mediated lysis of infected cells.

The humoral immune response is mediated by B cell-produced antibodies against HIV-1 antigens [14]. Non-neutralizing antibodies can control or eradicate a viral infection by multiple ways such as antibody-dependent cellular cytotoxicity (ADCC) and phagocytosis of infected cells. ADCC involves the activation of FcγRIIIA of NK cells by binding the Fc portions of antibodies bound to antigens expressed on the surface of target cells [15]. However, HIV-1-specific antibodies develop at an early stage of the infection but are unable to neutralise the virus due to acquisition of rapid sequence variation by virus [16].

DC (dendritic cell) plays a pivotal role in bridging the innate and adaptive immune responses against HIV-1, and the cross-talk between NK cell and DC is found to be essential for optimal NK cell activity. Priming of DC leads to NK cell activation and killing of immature DC (iDC) [17]. Activation is largely triggered by DC-secreted cytokines including IL-12 and IL-15. Thus, a perturbed NK–DC interaction results in poor anti-HIV activity [18]. In fact, HIV-1-infected individuals show a decrease in plasmacytoid DC number and IFN-α production that potentially interferes with the NK–DC interaction. In addition, HIV-1 patient's derived activated autologous NK cells are inefficient at lysing iDC [19]. Conversely, HIV-1-infected DC interactions with healthy control NK cells led to impaired receptor repertoire [20]. These studies suggest that NK–DC interactions play a key role in regulating each other's function and antiviral defence.

Implementation of highly active antiretroviral therapy (HAART) has significantly reduced HIV-1-associated mortality and morbidity across the globe, mainly by suppressing virus replication and $CD4^+$ T cell recovery. However, the poor immune reconstitution in the gut-associated lymphoid tissues (GALT) and persistent immune activation remain major therapeutic challenges despite effective HAART regimen. Although the majority of individuals respond to therapy, a significant population constituting around 20 % are unable to achieve optimal immune recovery referred as immunological non-responders. Thus, these patients are at higher risk of developing AIDS-like illness compared with responders. Despite improved prognosis in the era of HAART, non-AIDS related co-morbidities, such as cardiovascular diseases, remain one of the major therapeutic challenges.

## Mechanisms of NK cell control of HIV-1 infection

Over the years, seminal contributions have been made to our understanding of NK cells mediating immune protection against human viruses including, human cytomegalovirus (HCMV) [21], hanta virus [22], hepatitis-B virus (HBV) [23], hepatitis-C virus (HCV) [24] and HIV-1 [3]. Early evidence of antiviral NK cell activity was reported in murine cytomegalovirus (MCMV) infection [25], where virus-induced type-1 interferons (IFNs) produced by DCs resulted in NK cell-dependent protection [26]. As

mentioned above, NK cell effector functions are severely impaired, and viremia has been suggested as one of the key factors of cellular defects [27]. It is well appreciated that NK cells from HIV-1 patients respond poorly towards target cells and produce low levels of IFN-γ and Gr. In addition to lysis, NK cell production of C–C chemokine CCL3 (MIP-1α), CCL4 (MIP-1β) and CCL5 (RANTES) has been shown to block the entry of HIV-1 R5 strains [28, 29]. Therefore, less production of these molecules by functionally impaired NK cells may result in accelerated virus replication. In addition to controlling viral replication, NK cells can also kill non-infected $CD4^+$T cells, which start expressing NKp44L (a ligand of the activating NK receptor NKp44). $CD4^+$T cells expressing NKp44L became susceptible to NK lysis mediated by $NKp44^+$NK cells thereby playing a role in the $CD4^+$T cell depletion that happens throughout the HIV-1 disease progression [30].

ADCC plays a critical role in NK cell-mediated killing of HIV-infected cells. HIV-specific antibodies can trigger NK cell's activation by binding to both the HIV-1 antigens and FcγRIIIA leading to NK cell lysis of target cells. ADCC potentially offers an effective, adaptive immune response to HIV-1 infection, and broader ADCC responses may play a role in long-term control of HIV-1 disease progression [31]. ADCC has also been suggested as a key factor in HIV-1 protection conferred by EC owing to higher levels of ADCC antibodies than viraemic subjects [32]. Strong evidence in support of antibody-dependent NK cell-mediated protection against HIV-1 has recently been observed in Thai RV144 HIV-1 vaccine trials [33] where a majority of the treated individuals exhibited profound antiviral NK cell effector activity. Although ADCC is found to be an effective way of eliminating HIV-1-infected cells, still this is not always the case; very recently, a longitudinal study has shown diminished HIV-specific effector antibody responses, including ADCC in infected individuals receiving HAART [34]. This study suggests that despite successful treatment, therapeutic approaches may require improved antibody-mediated HIV-1 control.

## NK cell evasion by HIV-1

Similar to other human intracellular pathogens, HIV-1 has likewise evolved mechanisms to circumvent NK cell activity, including escape from ADCC owing to mutation in HIV Env glycoprotein (gp120 and gp41), epitope masking, trimerization of gp120/gp41 spikes and shedding of HIV-1 Env proteins [15, 35]. Although both $CD8^+$ T cells and NK cells have the ability to efficiently lyse HIV-1-infected cells, they experience great difficulty in cases where virus selectively downregulates surface HLA-A and HLA-B, while protecting the expressions of HLA-C and HLA-E molecules [36, 37]. Therefore, only those NK cells, which lack the inhibitory receptors specific for HLA-C and HLA-E, and HIV-1-infected cells with downregulated expressions of HLA-A and HLA-B, became susceptible to NK cell-mediated cytotoxicity [38]. Another mechanism involves downregulation of NKG2D ligands MHC class-related chain-A (MICA), -B (MICB) and HCMV UL-16 binding proteins (ULBPs) potentially by the HIV-1 protein Nef [39], thereby, preventing the binding of NKG2D receptors and their downstream signalling, a key step required for efficient NK cell killing.

In addition, HIV accessory protein Vpu has been suggested to influence the HIV-specific ADCC [40]. For example, Vpu downmodulates NK cell co-activating ligand NTB-A on the surface of infected $CD4^+$ T cells, thereby protecting the infected cell's lysis by NK cells [41]. Further, Vpu has been shown to downregulate and degrade tetherin, a cellular host restriction factor that enables the release of free virus aggregates [42, 43]. As a result, the recognition of these virus aggregates by HIV-specific antibodies and subsequent NK cell-mediated viral clearance is hampered [44]. In contrast to Vpu, HIV-accessory protein Vpr upregulates the expression of NK cell-activating ligands ULBP-1, -2 and -3 in HIV-1-infected primary $CD4^+$T lymphocyte, thereby enhancing their susceptibility to NK cell-mediated killing [45]. Another evasion mechanism involving Vpu and Nef proteins is the downregulation of PVR (CD155, Necl-5), the ligand for NK cell-activating receptor DNAM-1 (CD226), thereby, preventing NK cell-mediated lysis of HIV-1-infected $CD4^+$ T cells [46]. These studies explain how HIV-1 uses sophisticated strategies to circumvent antiviral immune response and ensures their survival in host body.

## NK cell subsets in HIV-1 infection

Based on surface CD56 density, NK cells are categorised into three distinct subsets: the cytokine producing $CD56^{bright}$ $CD16^-$ subset, the cytotoxic $CD56^{dim}$ $CD16^+$ subset, and a minor $CD56^{neg}$ $CD16^+$ NK cell subset with poor antiviral activity (Fig. 1). The figure shows an elaborated view of each subset in relation to HIV-1 pathogenesis.

## HIV-1 and $CD56^{bright}$ NK subsets

A subset of $CD56^{bright}$ NK cells represents approximately 5–10 % of the total peripheral blood NK cells. However, they record relatively higher frequency in secondary

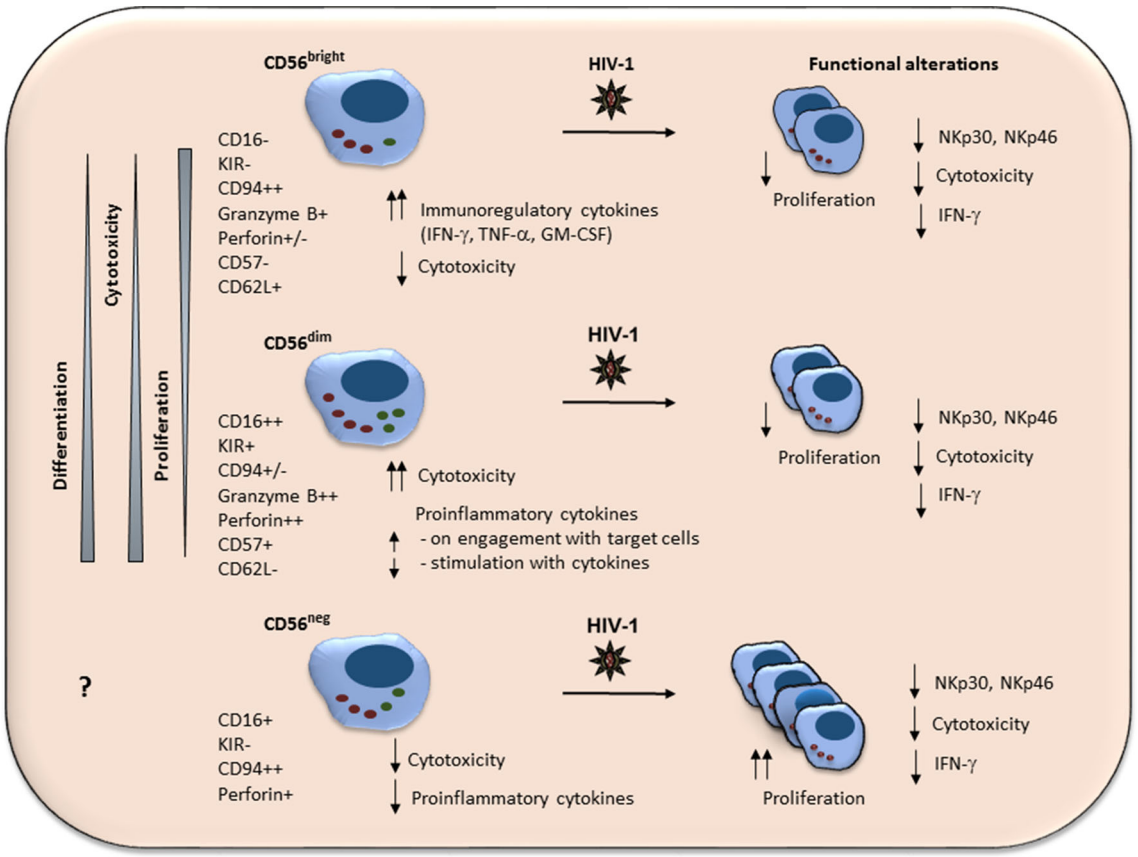


**Fig. 1** Diverse phenotypic and functional attributes of NK cell subsets in HIV-1 infection. Schematic representation of major human peripheral blood NK cell subsets, $CD56^{bright}$, $CD56^{dim}$ and $CD56^{neg}$, expressing different levels of antigens. Also, depicted are the expression levels and pattern of NK cell subset-specific markers, representing their differentiation and functional attributes. For example, the highly differentiated $CD56^{dim}$ population expresses higher levels of CD16, KIR, NKG2A, CD57 and Gr than less-differentiated $CD56^{bright}$ subset. In addition to expression of differentiation markers, the $CD56^{dim}$ subset is less proliferative but more cytolytic than $CD56^{bright}$ NK cells. During HIV-1 infection, there is redistribution of these subsets and the display of low levels of NCR associated with diminished cytotoxicity and IFN-γ production across the subsets. The unusual $CD56^{neg}$ subset expresses substantial levels of CD16 and Gr, but is functionally impaired in HIV-1 infection and more proliferative than $CD56^{dim}$ and $CD56^{bright}$ subsets. However, the differentiation pathway of $CD56^{neg}$ NK subset is not fully understood

lymphoid organs (SLO) such as lymph nodes. $CD56^{bright}$ NK cells are less cytotoxic but potent producers of immuno-regulatory cytokines. In addition to their low levels of CD16 and KIR, $CD56^{bright}$ NK cells express the inhibitory receptor CD94/NKG2A, the lymph-node-homing receptor CCR7 and L-selectin CD62L [47–49]. From the developmental point of view, $CD56^{bright}$ NK cells are considered precursors to highly differentiated $CD56^{dim}$ NK counterparts [50], and are suggested to pass through several intermediate stages comprising $CD16^{+}CD56^{bright}$ [51], $CCR7^{-}$ $CD56^{bright}$ [49], CD94-expressing $CD94^{bright}$ $CD56^{dim}$ [52] and $CD62L^{+}$ $CD56^{dim}$ cells [53], before acquiring the fully mature $CD56^{dim}$ NK phenotype (Fig. 1). Moreover, the environmental signalling either homeostatic and/or pathogen-induced pro-inflammatory, and receptor engagement might be the governing force in driving the NK cell differentiation and associated phenotypic diversity. Apart from secreting immuno-regulatory cytokines, $CD56^{bright}$ NK cells proliferate more in response to cytokine stimuli than their $CD56^{dim}$ NK counterparts, a feature of less-differentiated NK cells. The role of $CD56^{bright}$ NK cell in HIV-1 pathogenesis is less studied compared to $CD56^{dim}$ and $CD56^{neg}$ subsets. Clinical data support a sharp decline in the overall $CD56^{+}$ NK population accompanied by expanding $CD56^{neg}$ NK cells in early stage of infection, a phenomenon believed to be a compensatory mechanism to overcome the loss of $CD56^{+}$ cells [54].

One such report on impact of HIV-1 on the $CD56^{bright}$ NK cell subset has recently been reported from our laboratory, describing an altered phenotype with respect to the homing receptor CCR7 [49]. The chronically infected therapy naïve individuals show a significant loss of $CCR7^{+}$ $CD56^{bright}$ NK cells associated with relevant increased

$CCR7^-$ subpopulation. Interestingly, the loss of the $CCR7^+$ subpopulation positively correlates with HIV-1 viremia, and their frequency is moderately restored with HAART. These observations argued most likely downstream effects of chronic immune activation are caused by rapid HIV-1 replication. The role of $CD56^{bright}$ NK cells in antiviral host defence is also reported in HIV/HCV co-infection, where CD27-expressing $CD56^{bright}$ NK cells are suggested to resolve acute HCV infection in HIV-1 seropositive individuals [55] potentially via IFN-γ-mediated HCV suppression. Further investigations are required to understand the contribution of various $CD56^{bright}$ intermediate stages mentioned above, in human pathophysiology including acute and chronic HIV-1 infections.

## HIV-1 and $CD56^{dim}$ NK subsets

$CD56^{dim}$ NK cells constitute the major proportion (approximately 90 %) of the peripheral blood NK cells. Accumulating data support the linear differentiation of $CD56^{dim}$ NK cells from $CD56^{bright}$ precursors following a sequential and gradual process involving loss of surface CD94/NKG2A and gains in CD16, KIR and CD57 expressions [56] before achieving terminally matured status (Fig. 1). Functionally, $CD56^{dim}$ NK cells effectively lyse the virus-infected cell as they produce large quantity of executioner proteins, Gr and pfr. However, the notion that $CD56^{bright}$ NK cells are mainly cytokine producers and $CD56^{dim}$ NK cells act as killers may not hold true since both subsets produce IFN-γ. In fact, $CD56^{dim}$ subset has been shown to produce higher quantity of IFN-γ compared to $CD56^{bright}$ NK subset on engagement with target cells rather than with IL-2, IL-12, IL-15 and IL-18 stimulation [53]. As mentioned before, the overall loss of $CD56^+$ NK cells including $CD56^{dim}$ is suggested to be a consequence of ongoing virus replication, and this loss can be compensated by rapidly dividing aberrant $CD56^{neg}$ NK cells [57].

Our own laboratory has observed preferential loss of less-differentiated $CD56^{dim}$ NK cells, specifically those that produce low levels of IFN-γ in response to IL-12 and IL-18 [58]. Of note, the loss was restricted mostly to less-differentiated, $CD57^-$ and $CD57^{dim}$ subpopulations compared with $CD57^{bright}$ $CD56^{dim}$ NK subsets [59]. Furthermore, the increased levels of terminal differentiation marker CD57, along with KIR and Gr expressions on $CD56^{dim}$ NK, reflect the features exhibited by mature NK cells. However, the mechanisms of preferential loss of less-differentiated $CD57^-$ $CD56^{dim}$ NK cells during chronic HIV-1 infection are unknown. During the differentiation process, NK cells acquire CD57 and KIRs expressions to gain a mature phenotype [60]. Among them, $CD56^{dim}$ $CD57^+$ NK cells are found to exhibit better ADCC than $CD56^{dim}$ $CD57^-$ NK cells [59], suggesting again the superior functional attributes of mature NK cells. While educated $KIR3DL1^+$ NK cells show higher activation against autologous targets coated with HIV-1 antigens and antiviral antibodies compared with $KIR3DL1^-$ NK cells [61]. These higher capacities of $CD57^+$ and $KIR3DL1^+$ NK cells to exhibit elevated anti-HIV antibody-dependent activation suggest that education and differentiation contribute independently to ADCC-mediated NK cell activation [62]. The functional impairment of $CD56^{dim}$ NK cells has been demonstrated in NK cell–DC co-culture experiments. Studies show poor NK cell lysis, when healthy control $CD56^{dim}$ NK cells were co-cultured with HIV-1 viremic and aviremic-derived DCs, potentially due to defective NK–DC interaction [63], involving impaired maturation and cytokine production by iDC [64].

## HIV-1 and $CD56^{neg}$ NK subsets

The loss of $CD56^+$ NK cells during HIV-1 infection is associated with expansion of a poorly defined aberrant $CD56^-$ $CD16^+$ ($CD56^{neg}$) NK cell population [65], expressing low levels of natural cytotoxicity receptor, NKp30 and NKp46 [57] without changing NKG2D [66], reduced IFN-γ production, and impaired cytotoxicity [54] (Fig. 1). However, the data on inhibitory receptors are conflicting: one study reported increases expressions of KIR2DL2, 3 and LILRB1 [57], while another found lower KIR2DL1 expression on $CD56^{neg}$ NK cells [66]. Further, compared with $CD56^{dim}$ NK cells, $CD56^{neg}$ NK cells respond poorly to ADCC on stimulation [65]. However, both HIV-1 and HCV patient's derived $CD56^{neg}$ NK cells have been shown to produce significant amount of antiviral chemokine MIP-1β [66, 67]. In contrast to $CD56^{dim}$ and $CD56^{bright}$ NK cells, the lack of major NK cell lineage-specific markers on $CD56^{neg}$ NK cells hinders our understanding of their origin and developmental pathways. Further, sharing of characteristic phenotypes of both less-differentiated (low CD57 expression and high proliferative capacity) and highly differentiated $CD56^{dim}$ NK cells (low CD94/NKG2A and elevated KIR expressions) on $CD56^{neg}$ NK cells make them phenotypically more complex to define their origins [57, 65, 68]. Nonetheless, based on phenotypic and functional characteristics, one may argue that $CD56^{neg}$ cells might have originated from early $CD56^{dim}$ stage. However, further studies are required to specifically address this issue.

Under physiological conditions, $CD56^{neg}$ NK cells represent only a minor subset. However, their population increases during chronic viral infections including HIV-1 [54, 57, 65, 68, 69] and HCV [67, 70]; therefore, their

relevant increases may act as a potential pathophysiological indicator. Impaired HIV-1-associated $CD56^{neg}$ NK cell functional defects were also seen in ex vivo co-culture experiments with DC [63], reflecting their inability to communicate effectively with DC. These alterations may not be considered a consequence of HIV-1 infection since the occurrence of productive infection of NK cell is a rare event, except in one study [71]. Importantly, $CD56^{neg}$ NK cell subsets show substantial heterogeneity. For example, activated $CD7^{+}$ $CD56^{neg}$ NK cells, but not $CD7^{-}$ $CD56^{neg}$ NK cells, expressing CD95, KIRs and NKG2A/C, selectively expand during HIV-1 infection [72]. In addition to these, two additional subpopulations reported to emerge during infection are $CD122^{-}$ $CCR7^{+}$ and $CD122^{+}$ $CCR7^{-}$ subsets. Of note, the latter subset expands significantly and shows positivity for the terminal differentiation marker CD57, reflecting their closeness with mature $CD56^{dim}$ NK cells [69].

As mentioned before, the expansion of $CD56^{neg}$ NK cells during HIV-1 infection has been suggested to be a mechanism to compensate for the loss of $CD56^{+}$ NK cells in order to maintain overall NK cell homeostasis in infected individual [54]. Moreover, the rapid $CD56^{neg}$ NK cell expansion has also been argued as a consequence of high viremia, since both parameters strongly correlate with each other but not with virally suppressed LTNPs [57]. In this context, HAART-mediated suppression of HIV-1 viremia causes considerable reduction of $CD56^{neg}$ NK cells, supporting the notion that viremia indeed remains a major inducing factor for aberrant cellular phenotype. Similarly, antiretroviral and pegylated IFN plus ribavirin treatment of HIV/HCV co-infection has led to reduced numbers of aberrant $CD56^{neg}$ NK cells [70].

Apart from the reduced CD56 expression during infection, Siglec-7, an inhibitory C-lectin-type molecule has been suggested as one of the early surface markers downregulated with HIV-1 infection [73]. Interestingly, Siglec-7 expression remains suppressed throughout the course of infection, and their regulation substantially relies on viremia, as LTNPs and ECs display normal cell distribution. All these studies, therefore, describe accumulation of an aberrant and "anergic" $CD56^{neg}$ NK cell population in response to $CD56^{+}$ NK cell loss, which could be responsible for overall NK cell redistribution and associated dysfunctions. Considering the immune correlates of protection against HIV-1, our laboratory has recently described a new polyfunctional $CD8^{+}$ NK cell phenotype in a large cohort of HIV-1 positive individuals, which inversely correlates with the diseases progression [74]. Therefore, a higher frequency of these cells may reflect an improved antiviral NK cell activity, and this may potentially serve as a non-genetic determinant of HIV-1 disease progression.

## Changes in the NK cell receptor repertoire in HIV-1 immuno-pathogenesis

Given that NK cell effector function depends on the balance between activating and inhibitory receptors, it is largely unknown how the relative NKR alterations in HIV-1 infection affect NK cell antiviral activity. Discussed below are the major NKRs and their potential role in HIV-1 immuno-pathogenesis.

## Natural cytotoxicity receptors (NCRs)

NCRs are germ-line-encoded major NK cell-activating receptors comprising NKp30, NKp44, and NKp46. Binding of these receptors to their putative ligands mostly expressed on stressed, tumour and virus-infected cells triggers lysis of these cells [75, 76]. Some of the important human viral ligands recognised directly by NCRs are summarised (Table 1). The vital role of NCRs in antiviral host defence has been reported both in animals and humans. For instance, NKp46 is found to be essential to clear influenza virus in mice [77]. NCRs exhibit a certain degree of redundancy as they can recognise the same ligand of a different viral origin. For example, influenza, Sendai, and New Castle disease virus-derived haemagglutinin (HA) and HN (HA-neuraminidase) have affinity towards both NKp44 and NKp46 receptors [78–80]. Contrary to this, despite being an activating receptor, NKp30's engagement with HCMV-pp65, Pox and vaccinia-derived HA inhibits NK cell activity [81, 82]—a viral evasion mechanism to avoid lysis of infected cells. That means NK cell binding of viral ligands is more or less dependent on individual engaging NCR and not necessarily all NCRs deliver activation signals.

Antiviral activity by NCR has also been shown in chronic hepatitis C virus (HCV) infection, where $NKp46^{hi}$ NK cell subset suppresses virus replication in vitro [83]. The anti-HCV characteristics of these cells were due to high levels of IFN-$\gamma$ secretion and increased cytolytic activity. Accumulating data support abnormal NCR repertoire associated with impaired cytotoxicity and immunoregulatory functions in HIV-1 infection [73, 84]. Notably, HIV-1 viremia greatly impacts NCR-triggered NK cell effector function as observed in HIV-1 viremic individuals, who display a marked reduction in NKp30 and NKp46 receptor associated with poor cytotoxicity and IFN-$\gamma$ production compared with aviremic individuals [27, 57, 85]. This is supported by another study where LTNP and EC, with suppressed viremia, successfully maintain NCR and cellular functionality in contrast to antiretroviral-treated aviremic progressors [86]. These studies suggest that active viral replication profoundly impacts NCR

**Table 1** NK cell-activating receptors and their identified viral ligands

| Receptors | Virus and their ligands | Activation | Refs. |
|---|---|---|---|
| NKp46 (NCR1/CD335) | Influenza, New Castle disease, Pox and Sendai virus HA and HN | Yes | [77, 79, 81] |
| NKp44 (NCR2/CD336) | Influenza, New Castle disease and Sendai virus HA and HN | Yes | [77, 78, 80] |
| NKp30 (NCR3/CD337) | HCMV-pp65, Pox and Vaccinia virus HA | No | [81, 82] |
| NKG2D | HCMVMIC -A,-B and ULBPs | Yes | [111] |

*HA* haemagglutinin, *HN* HA neuraminidase, *MIC -A* and *-B* MHC class I chain-related protein (MIC)-A and -B, *ULBPs* UL16-binding proteins

regulation and their downstream effector function. In contrast, two recent studies have demonstrated down-regulation of NKp46 receptors on NK cells resulting in impaired cytolytic activity and diminished innate immunity during chronic viral infection [87, 88].

## Killer cell immunoglobulin (Ig)-like receptors (KIRs)

KIRs are differentially expressed on NK cell subsets [89], and every single human NK cell expresses at least one to several MHC-1-specific NK cell receptors (on average 2–9 different KIRs and/or KLRs per cell) resulting in a diverse NK cell repertoire [90–92]. Both KIR and HLA polymorphisms play a central role in defining NK cell-mediated outcome of HIV-1 disease. One of the early reports of KIR:HLA interaction on virus control demonstrates resolution of HCV infection in individuals positive for KIR2DL3 and homozygous for HLA-C1 [24], while individuals, expressing KIR3DS1 and positive for HLA-Bw4-80I, are protected against developing HCV-induced hepato-cellular carcinoma [93]. Furthermore, the KIR and HLA combination also plays a role in determining the HIV-1 transmission between sexual partners. In this regard, inhibitory KIR/HLA incompatibility appears to confer protection against HIV-1 transmission [94]. Moreover, the viral peptide selectivity of KIRs is found to be much broader compared with adaptive T cell receptors [95] since KIRs recognise peptide motifs, rather than individual peptides. This means that KIR binding essentially depends on the sequence and structure of epitope presented by HLA class-1 molecules. In addition, KIR-associated amino-acid polymorphism also contributes to NK cell control of HIV-1. For example, HIV-1-selected sequence polymorphism in KIR2DL2 confers strong binding with infected $CD4^+$ T cell, resulting in NK cell inhibition [96].

Recent population genetic studies have revealed the influence of HLA–KIR association in HIV-1 disease and clinical outcome (Table 2). For example, individuals positive for HLA-Bw4-80I show delayed progression towards AIDS than those without this allele, mostly due to their interaction with both activated KIR3DS1 and inhibitory KIR3DL1 receptors on NK cells [97, 98]. Notably, NK cells positive for KIR3DL1 confer better cellular function in individuals with HLA-Bw4 than HLA-Bw6 homozygous alleles [99, 100], while the protective effect conferred by KIR3DS1-expressing NK cells [101–103] is due to their efficient capability to suppress HIV-1 replication [104]. Further, HLA-C-educated NK cells positive for inhibitory KIR2DL1-3, appear to modulate NK cell functionality during primary HIV-1 [105]. In addition to HLA–KIR interaction, KIR copy number greatly impacts the outcome of the disease. For example, a recent study has shown strong association of activating receptor KIR2DL4 copy number with better $CD4^+$ T cells recovery in SIV-infected Mamu-A*01-negative rhesus macaques [106], suggesting KIR copy number is a strong protective determinant of the disease. In the same line, a genome-wide screening of structural variants has demonstrated association of high KIR3DS1 copy number with lower viral set point in the presence of a putative ligand, while NK cells from individual with high copy number of KIR3DL1 in the presence of KIR3DS1 and ligand show efficient inhibition of HIV-1 replication [107].

As discussed above, while the amino acid sequences do influence the HLA–KIR interaction, recent studies have revealed how a minimal sequence variability within HLA-C allele-restricted HIV-1 Gag24 epitopes can significantly affect NK cell functions. Recognition of HLA-C03:04- and HLA-Cw*0102-restricted peptide by inhibitory KIR2DL1 exhibits reduced NK cell functions [108, 109]. Interestingly, NK cells that are educated through KIR3DL1 mediate potent anti-HIV ADCC against autologous HLA-$Bw4^+$ target cells. However, in HIV-1 infection, no functional differences were observed between $KIR3DL1^+$ and $KIR3DL1^-$ NK cells in HLA-$Bw4^+$-infected individuals, suggesting abrogation of functionally competent educated NK cells may have resulted in poor ADCC, and thus contributed to HIV-1 disease progression [61]. Taken together, the above studies suggest that NK cells' ability to control HIV-1 disease is significantly dependent on the HLA allele and to a certain extent on KIR polymorphism of the host.

**Table2** Influence of KIR–HLA interaction on chronic viral diseases

| KIR | HLA | Function | Virus | Consequence | Refs. |
|---|---|---|---|---|---|
| KIR3DS1 | HIA-Bw4-80I | Activating | HIV-1 | Delay progression to AIDS | [97] |
| KIR3DL1 | HUVBw4-80I | Inhibitory | HIV-1 | Delay progression to AIDS | [98] |
| KIR3DS1 | HLA-Bw4 | Activating | HIV-1 | Protection from infection | [101] |
| KIR2DL3 | HIA-C1 | Inhibitory | HCV | Infection resolution | [24] |
| KIR3DS1 | HLH-Bw4-BDI | Activating | HCV | Protection from HCC | [93] |

*KIR* killer immunoglobulin-like receptor, *HLA* human leucocyte antigen, *HCC* hepatocellular carcinoma

## C-type lectin-like receptors

Most characterised human C-type lectin-like receptors mainly include activating NKG2D and CD94/NKD2C, and inhibitory CD94/NKG2A and NKR-P1A (CD161). Human CD94/NKG2A and CD94/NKG2C receptors are heterodimers that recognise non-classical, loaded HLA-E molecules and deliver inhibitory and activating signals to NK cells, respectively. NKR-P1A (CD161) binds to a non-MHC-coded ligand and lectin-like transcript-1 (LLT-1) [110]. Among all the C-type lectin-like family members, NKG2D is the most studied receptor, since many of its ligands including MICA, MICB, and ULBP 1–4 of HCMV have been identified [76, 111]. Like other intracellular human pathogens, HIV-1 employs well-developed evasion strategies to circumvent the immune system. In this context, HIV-1 has been shown to downregulate NKG2D ligands on virus-infected cells potentially via protein Nef to evade NK cell-mediated killing [39]. Similarly, soluble NKG2D ligands present in the plasma of HIV-1-infected individuals has been demonstrated to suppress NKG2D-mediated NK cell impairment [46]. Nonetheless, modulation of NKG2D ligands by viral proteins Vif, Nef, Vpu significantly contribute to poor recognition of infected cells, and thus in poor target cell lysis. Moreover, it is also believed that certain NKG2D ligands are induced by HIV-1-infected $CD4^+$ cells and that NK cells effectively lyse them. In this regard, HIV-1-infected $CD4^+$ T cells have been shown to express elevated NKG2D ligand via Vpr-APOBEC3G interaction-induced DNA-damage response and are effectively lysed by NK cells [112].

## Emerging concept of memory-like NK cells

Generation and persistence of memory T and B cell lymphocytes have been extensively studied in various animal and human infection models [113], but in recent years, several studies have come up with 'memory-like' properties of NK cells in pathogenic and non-pathogenic models. In this context, one of the early observations described hapten-specific liver NK cell recall response to ear swelling in T- and B-cell-deficient mice [114]. In another murine infection model, MCMV m157-specific clonal expansion and recall response were observed in $Ly49H^+$ NK cells [59, 115]. Later, several groups have reported memory-like NK cell behaviour in other viral infections, including chronic hepatitis viruses [116], Hanta [22], and Chikungunya [117] in humans, and vaccinia [118], HSV [119] and influenza [120] in mice. However, due to lack of concrete evidence, this phenomenon is yet to be fully proven in humans. In addition to in vivo detection of memory-like NK cells, NK cells pre-activated with IL-12/IL-15 and IL-18 have been shown to induce memory-like phenotype in re-stimulation experiments as determined by high IFN-γ production [121]. Similar observations were shown in an adoptive transfer model of cytokine pre-activated NK cells into mice following re-stimulation experiments [122]. Currently, we do not have reports of memory-like like NK cells in HIV-1 infection, an area that needs thorough investigation. NK cell memory is gaining immense interest pertaining to their generation in acute infection and long-term persistence in pathogenic conditions. Whether innate lymphocytes such as gamma-delta T (γδ), natural killer T (NKT) and mucosal-associated invariant T (MAIT) cells possess this property is an interesting area that needs to be investigated.

## Concluding remarks

The increasing body of evidence strongly supports the role for NK cell-mediated immune protection against HIV-1. Identification of new KIR alleles through population-based studies has significantly improved our understanding of HIV-1 disease outcome. However, a detailed mechanistic aspect of HLA–KIR associations could prove beneficial in designing NK cell-based immunotherapy. With the emergence of memory-like NK cells, our focus should be directed towards potential implications in immunotherapy against viral infection. Perhaps non-human primate (NHP) models of SIV/SHIV infection can provide better insight into our understanding in designing NK cell-targeted vaccines. The use of sophisticated tools such as mass cytometry-based investigation on NK cell may provide in-

depth cellular and molecular insights on roles in human pathophysiology. Certainly, more experimental models and investigations are required to understand the mechanisms that translate the NK cell function which protects from HIV-1 infection and suppression of disease progression.

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