Natural killer cells in antiviral immunity

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Abstract | Natural killer (NK) cells play an important role in innate immune responses to viral infections. Here, we review recent insights into the role of NK cells in viral infections, with particular emphasis on human studies. We first discuss NK cells in the context of acute viral infections, with flavivirus and influenza virus infections as examples. Questions related to activation of NK cells, homing to infected tissues and the role of tissue-resident NK cells in acute viral infections are also addressed. Next, we discuss NK cells in the context of chronic viral infections with hepatitis C virus and HIV-1. Also covered is the role of adaptive-like NK cell expansions as well as the appearance of $CD56^-$ NK cells in the course of chronic infection. Specific emphasis is then placed in viral infections in patients with primary immunodeficiencies affecting NK cells. Not least, studies in this area have revealed an important role for NK cells in controlling several herpesvirus infections. Finally, we address new data with respect to the activation of NK cells and NK cell function in humans infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) giving rise to coronavirus disease 2019 (COVID-19).

Antibody-dependent cellular cytotoxicity (ADCC). A mechanism by which natural killer cells, via their Fc receptor CD16, kill a target cell whose membrane-surface antigens have been bound by a specific antibody.

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Almost 50 years ago, a small number of research groups started to observe unexpected spontaneous cytotoxic activities among lymphocytes. While these were at first considered to be merely annoying background phenomena, subsequent studies led to the realization that a new subpopulation of lymphocytes was responsible for this activity[1]. The cells were termed 'natural killer (NK) cells'. Over the years, insights into the molecular specificity of NK cells in terms of target cell recognition[2–4] and into their role in controlling viral and intracellular bacterial infections and tumours, as well as insights into their ability to regulate other immune cells, have put NK cells at the forefront of modern immunology[5]. Today, they are used as therapeutic agents in the treatment of human malignant diseases[6], and strategies for using them therapeutically against viral diseases, including coronavirus disease 2019 (COVID-19), are currently being explored[7].

NK cells are best characterized by their cytotoxic function and their ability to produce cytokines upon stimulation[5]. With respect to their cytotoxic potential, they target infected, transformed and stressed cells. In this way, they not only eliminate unwanted cells but also contribute to cellular homeostasis[8]. The conventional known anatomical site of NK cell production is the bone marrow, where interactions with other cellular components, cytokines and soluble molecules support and drive NK cell development (reviewed in REF.[9]) (BOX 1). Most of the current knowledge of human NK cells has arisen from studies of NK cells in peripheral blood and secondary lymphoid organs. Recently, however, there has been increased interest in NK cells residing in specific tissues[10] (so-called tissue-resident NK cells) (BOX 2).

In humans, mature NK cells are traditionally identified as $CD3^-CD56^+$ lymphocytes. They were long thought to represent a homogeneous population of cells. However, in line with adaptive lymphocytes, we now know that NK cells undergo a well-defined differentiation process involving distinct phenotypic and functional changes (BOX 1).

NK cell function is regulated by a large number of germline-encoded receptors[11,12]. Upon viral infection, host cells may become susceptible to NK cell-mediated recognition through a variety of mechanisms that may include upregulation of self-encoded molecules induced by the infection and/or a concomitant cellular stress response that binds activating NK cell receptors such as natural cytotoxicity receptors (NKp30, NKp44 and NKp46)[13], C-type lectin-like receptors (for example, NKG2D and NKp80)[14] and co-activating receptors (for example, DNAM1 and CD2)[12]. In parallel, contributing to increased target cell susceptibility is the often observed downregulation of MHC class I ligands for inhibitory receptors[15]. Importantly, NK cells can also eliminate virus-infected cells via CD16-mediated antibody-dependent cellular cytotoxicity (ADCC)[16]. Finally, NK cell activity is modulated by cytokines, including, but not limited to, the activating cytokines IL-2, IL-12, IL-15, IL-18 and type I interferons, which can be produced by virally infected cells or activated antigen-presenting cells[17]. Many of these cytokines, alone or in combination, promote NK cell survival, proliferation, cytotoxicity and cytokine production, including interferon-γ (IFNγ) production[18]. Through these mechanisms, NK cells are uniquely equipped to sense and quickly respond

Box 1 | **Human NK cell differentiation**

Natural killer (NK) cells undergo the final steps of their development in secondary lymphoid organs, resulting in mature $CD56^{bright}CD16^{-}$ NK cells that enter the circulation[146]. $CD56^{bright}$ NK cells are efficient in producing cytokines but have limited cytotoxicity. They can further differentiate into $CD56^{dim}CD16^{+}$ NK cells, the NK cell population most dominant in peripheral blood. Compared with $CD56^{bright}$ NK cells, peripheral blood $CD56^{dim}$ NK cells are a highly diverse population of immune cells[93] that undergo a continuous IL-15-driven differentiation process during their lifespan[34]. This differentiation is associated with changes in cell-surface phenotype, including loss of expression of CD94, NKG2A and CD62L (also known as L-selectin), as well as induction of killer cell immunoglobulin-like receptor (KIR) and CD57 expression[34,36,147,148]. On a functional level, less differentiated $CD56^{dim}$ NK cells are more highly proliferative and more responsive to cytokine stimulation, whereas highly differentiated $CD56^{dim}$ NK cells lose their proliferative capacity and instead become more specialized with regard to cellular cytotoxicity and antibody-dependent cellular cytotoxicity[34]. A parallel pathway of NK cell differentiation occurs in non-lymphoid peripheral tissues (reviewed in REF.[10]) (BOX 2). Here, it is instead the less differentiated $CD56^{bright}$ NK cells that are thought to enter tissue parenchyma and, in response to the local microenvironment, likely differentiate into site-adapted cells[50]. Understanding NK cell differentiation, the factors driving it and the functional specialization of the discrete differentiation stages provides a framework for subsequently interpreting NK cell antiviral immunity.

to viral infections[7]. In this respect, they often act in concert with other host immune responses in mediating antiviral immunity.

Here, we review recently obtained knowledge with regard to human NK cells in antiviral immunity. We address NK cell responses to typical acute viral infections. In many situations, these trigger activation of circulating NK cells and their homing to affected tissues. In parallel, tissue-resident NK cells may also become activated. With respect to chronic viral infections, NK cells contribute to viral control. We describe how such infections may lead to 'adaptations' in the NK cell repertoire, likely contributing to their maintenance of viral control but possibly also representing a consequence of exhaustion in some cases. Impairment in NK cell-mediated viral control is seen in patients with inborn deficiencies affecting NK cell development and/or function. Finally, we pay specific attention to the very recently developing knowledge with respect to vigorous NK cell activation in human severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and COVID-19.

NK cells in acute viral infections

NK cells respond vigorously to several acute viral infections, exemplified in the following subsections by acute infections with flavivirus and influenza virus (both RNA viruses). In many situations, this triggers activation of circulating NK cells and their homing to affected tissues. In parallel, specific tissue-resident NK cells may also become activated. Critical to all host responses to acute viral infections is the balance between possible acute antiviral mechanisms and responses contributing to tissue damage and immunopathology.

Flavivirus infections. Flaviviruses, including dengue virus (DENV), West Nile virus, Japanese encephalitis virus, tick-borne encephalitis virus, yellow fever virus and Zika virus, are major emerging human pathogens, affecting millions of individuals worldwide. Flaviviruses elicit haemorrhagic fever-like syndromes and central nervous system-associated diseases in infected hosts[19]. Effective vaccines exist for some of these viral infections. With a few exceptions, there is a lack of knowledge with respect to which host cells are infected by flaviviruses in humans and whether they are targets of NK cell-mediated responses. Innate immune responses elicited by flaviviruses have been studied extensively in animal models, in particular DENV and Zika virus infections[20]. However, fewer studies have focused specifically on NK cells in these acute viral infections, with influenza virus infections being an exception, as reviewed in the next subsection. In humans, NK cell responses to acute flavivirus infection have been addressed in clinical studies, particularly in DENV infections[21,22]. While clinical studies performed to date have differed in their set-up, some common observations have been made with respect to NK cell responses in different flavivirus infections. In several studies, both $CD56^{bright}$ NK cells and, in some studies, subpopulations of less differentiated $CD56^{dim}$ NK cells (BOX 1) have been reported to respond vigorously to infection[22–25]. Type I and type III interferons likely contribute to the NK cell responses observed in several flavivirus infections, including yellow fever virus infection[23]. Responses occur irrespective of NK cell education, and no evidence of specific adaptive-like NK cell responses has been observed[22,23]. Concomitant with the increase in NK cell activation, increased levels of various cytokines, including IL-12, IL-15, IL-18, IFNγ and tumour necrosis factor (TNF), have been detected in flavivirus-infected patients[22,23,25]. In DENV infections, increased IL-18 levels in plasma and in induced skin blisters, as well as concomitant signalling downstream of IL-18 receptor in NK cells, have suggested an IL-18-dependent mechanism in driving the proliferative NK cell response[22]. This proliferation might occur via nuclear factor-κB (NF-κB), AKT, activating transcription factor 2 (ATF2) and forkhead box protein O3 (FOXO3) or indirectly via IL-18-induced upregulation of CD98 and consequently amino acid-induced activation of mechanistic target of rapamycin (mTOR)[22]. NK cell-produced IFNγ, on the other hand, has in other studies been suggested to be important for control of DENV infection[26]. These and other related studies illustrate the dynamics and specifics of the early NK cell response during prototype acute flavivirus infections in humans. How NK cells contribute to viral clearance in experimental and human flavivirus infections remains to be explored in further detail.

Influenza virus infections. Influenza viruses cause yearly global epidemics, with up to a billion infections annually[27]. While vaccines limit their spread, they do not prevent all infections. Typically, the virus infects lung epithelial cells. Like the situation in many viral infections, including SARS-CoV-2 infection (discussed in more detail below), infection with influenza virus can lead to excessive inflammation and tissue damage. Although NK cells are part of this response, their contribution to host defence against influenza A virus (IAV) is not fully understood. Upon IAV infection in mice, increased accumulation of NK cells is observed in the lungs[28]. While local proliferation of pulmonary

NK cell education
The process involving acquisition of functional competence in natural killer (NK) cells, often mediated by interactions between inhibitory receptors and the corresponding ligands.

Adaptive-like NK cell
A subset of expanded terminally differentiated natural killer (NK) cells that, in the context of human cytomegalovirus infection, is characterized by expression of NKG2C.

Box 2 | **Human tissue-resident NK cells**

Over the last decade, our knowledge of the presence, development, differentiation and function of natural killer (NK) cells residing in peripheral tissues has increased dramatically (reviewed in REF.[10]). It is now clear that most human organs, both lymphoid and non-lymphoid, contain tissue-resident NK cells expressing canonical tissue-residency markers such as CD69, CD103 and CD49a, all of which are functionally involved in retaining the cells in tissues[50,149–151] and have been shown to, de facto, associate with tissue residency in human solid organ transplantation studies[152,153]. The ontogeny of tissue-resident NK cells remains elusive. However, since their phenotype is in many respects (except for uterine NK cells) similar to that of blood CD56^{bright} NK cells, it is possible that they originate from CD56^{bright} NK cells that get cues to enter tissue microenvironments[10]. Alternatively, dedicated precursors may exist that seed peripheral tissues[154]. With their phenotype more resembling less mature CD56^{bright} NK cells, tissue-resident NK cells are in general less cytotoxic but more efficient in producing cytokines such as interferon-γ. While many human peripheral tissues contain a sizeable population of tissue-resident NK cells, mouse peripheral tissues such as the liver, salivary glands and uterus are instead populated by NK1.1^{+}CD49a^{+}DX5^{-} cells referred to as group 1 innate lymphoid cells (ILC1s). Yet, ILC1s likely represent the mouse counterpart of human tissue-resident NK cells[155].

NK cells may account for part of this response, most of the NK cell accumulation in the lungs and airways during IAV infection has been suggested to be due to NK cell recruitment that is partly dependent on CXC-chemokine receptor 3 (CXCR3) and CC-chemokine receptor 5 (CCR5), respectively[28]. Several NK cell natural cytotoxicity receptors have been implicated in functional activation of NK cells following influenza virus infection by binding to influenza virus haemagglutinin[29]. However, the degree to which NK cells contribute (if at all) to influenza virus elimination in this context is less clear. In humans, peripheral blood CD56^{bright} and CD56^{dim} NK cells have been shown to be primed during acute IAV infection[30]. Furthermore, in peripheral blood, a small subset of CD16^{-}CD49a^{+}CXCR3^{+} NK cells has been identified, with CD49a and CXCR3 potentially promoting homing to and tissue retention in the lungs during acute infection. At the primary site of infection, it is not fully clear how circulating and tissue-resident NK cells contribute to possible antiviral immunity or to pathology, such as by killing bystander cells[30]. Finally, studies in humans and macaques have demonstrated that cross-reactive antibodies elicited during seasonal influenza virus infections facilitate NK cell-mediated ADCC with regard to infected target cells[31,32]. This ADCC-mediated response has been correlated with reduced disease severity. In infections such as influenza virus infection, more research is clearly needed to better delineate and discriminate between possible protective antiviral responses and those leading to excessive inflammation and tissue damage.

NK cell activation in acute infections. The acute NK cell response to infection with flaviviruses, including tick-borne encephalitis virus, DENV and yellow fever virus, as well as SARS-CoV-2 (discussed in detail below), is to a large degree confined to less differentiated CD56^{bright} and CD56^{dim} NK cells[22,23,25,33] (BOX 1). Since these cells are more responsive to cytokines such as IL-12, IL-15, IL-18 and type I interferons, probably in part because of higher expression of receptors for these cytokines[22,34,35], it is likely that early NK cell activation is cytokine driven and less reliant on receptor–ligand interactions (FIG. 1).

JAK–STAT pathways
Signalling pathways involving Janus kinases (JAKs) and signal transducers and activators of transcription (STATs) downstream of many cytokine receptors, including those for IL-2, IL-12, IL-15 and type I interferons, but not IL-18, important in natural killer cell activation, proliferation and survival.

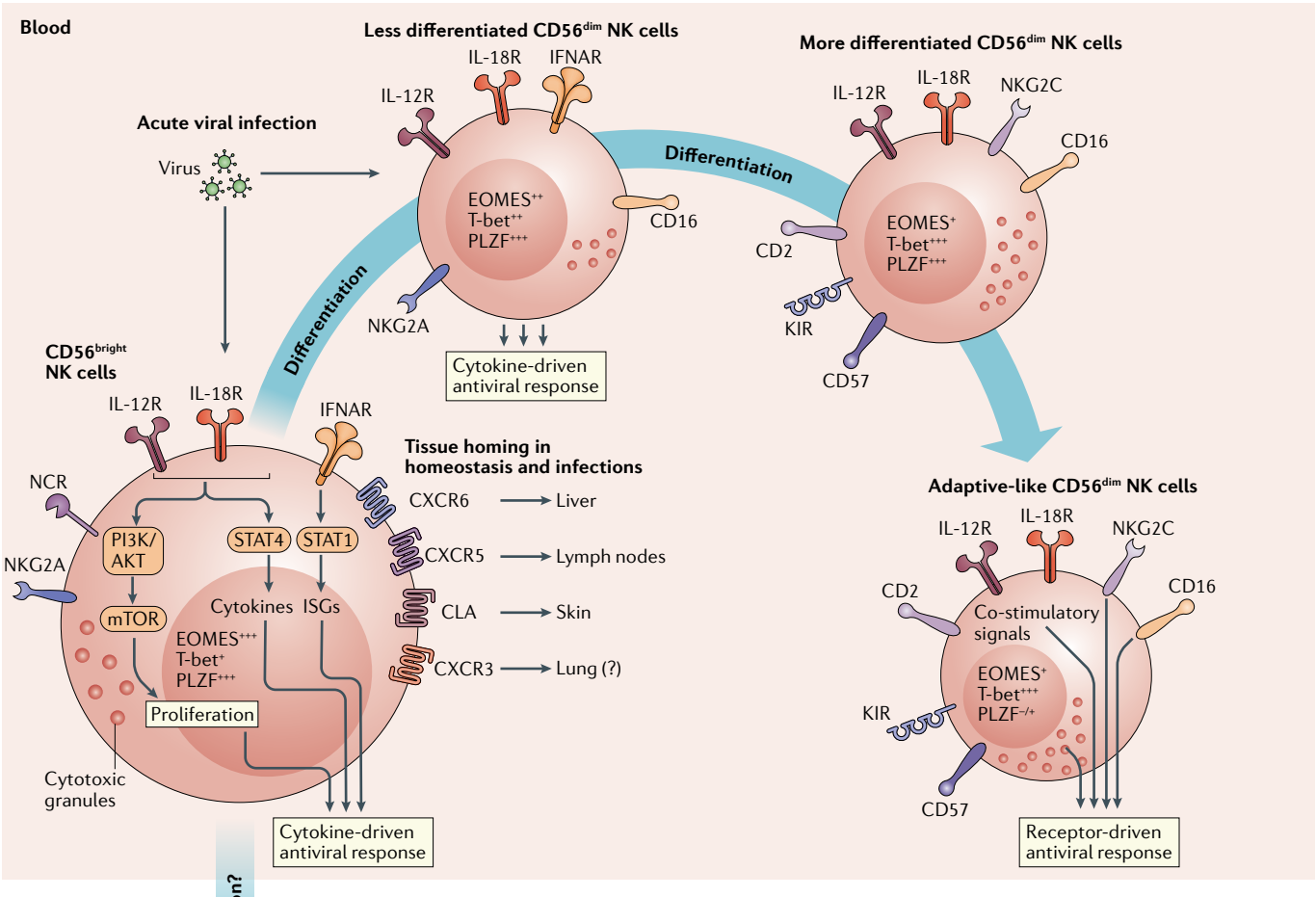
This results in stronger downstream signalling via signal transducer and activator of transcription 1 (STAT1), STAT4 and the PI3K–AKT–mTOR pathways in less differentiated NK cells[22,36,37], in which mTOR, via the IRE1α–XBP1–MYC axis, is crucial for NK cell proliferation following acute activation[38]. Cytokine signalling via JAK–STAT pathways in NK cells was recently comprehensively reviewed elsewhere[39]. For induction of early NK cell activation, type I interferon signalling via STAT1 and IL-12 plus IL-18 signalling via STAT4 result in strikingly different interactions with the NK cell epigenetic landscape[40]. More specifically, STAT1 and STAT4 can exhibit antagonistic functions on their downstream networks early after NK cell activation, including STAT4 actively suppressing STAT1 expression[40]. Furthermore, whereas STAT4 binding resulted in increased chromatin accessibility, STAT1 interacted more with promoter regions[40]. Although more long-lasting effects of these alterations in the chromatin landscape remain elusive, it is possible that remodelling via IL-12 plus IL-18 and STAT4 could have more permanent effects as STAT4 signalling is important for the generation of both adaptive-like and cytokine-induced memory-like NK cells[41,42].

A recent study by Sciumè and colleagues looked into the molecular mechanism of early NK cell activation in response to cytokines[43]. Analysis was focused

Fig. 1 | **NK cell differentiation as a framework to understand NK cell antiviral immunity.** The differentiation status of human natural killer (NK) cells determines their functional response in viral infections. CD56^{bright} NK cells and less differentiated CD56^{dim} NK cells express high levels of NKG2A, natural cytotoxicity receptors (NCRs; NKp30, NKp44 and NKp46) and proinflammatory and antiviral cytokine receptors (IL-12 receptor (IL-12R), IL-18R and type I interferon receptor (IFNAR)). During acute viral infections, with ensuing production of IL-12, IL-18 and type I interferons by other immune cells, less differentiated NK cells are the main NK cell subset to respond. As indicated by black arrows, cytokine stimulation leads to signalling via signal transducer and activator of transcription 1 (STAT1) and STAT4 and PI3K–AKT–mTOR pathways to rapidly induce NK cell cytokine production and proliferation. With increased maturation, circulating CD56^{bright} NK cells undergo a phenotypic shift and their responsiveness during infection becomes altered. As a consequence, more differentiated NK cells are more prone to respond to cytomegalovirus, leading to the expansion of adaptive-like NK cells that express high levels of killer cell immunoglobulin-like receptors (KIRs) and NKG2C. Circulating CD56^{bright} NK cells might also home to tissues, giving rise to tissue-resident NK cells. Their role in the early response to infection remains elusive. However, liver-resident NK cells have been shown to mediate antigen-specific antiviral responses. Alterations in transcription factor expression during NK cell differentiation are indicated within the respective cell nuclei, and a summary of phenotypic changes during NK cell differentiation is depicted in the table. For some receptors, the expression depends on the tissue microenvironment and specific subsets studied and will deviate from what is indicated here (denoted by the asterisk). CLA, cutaneous lymphocyte-associated antigen; HAV, hepatitis A virus; HBV, hepatitis B virus; ISG, interferon-stimulated gene; NA, not available.

on regions surrounding genes highly induced on early NK cell activation and revealed de novo chromatin and enhancer remodelling mediated by STAT4. These de novo enhancers were further repurposed to be used not only by STATs but also by the lineage-defining transcription factor T-bet[43]. Thus, although T-bet and other lineage-defining transcription factors were once considered pioneers for determining the shape of the epigenetic landscape that signal-regulated transcription factors act on, this instead suggests that in acute activation of NK cells STATs will remodel chromatin accessibility and invite lineage-defining transcription factors to participate in novel ways in the acute response.

NK cell homing in acute infections. Since many viruses have narrow tropisms, infections often originate at the site of entry before spreading locally and/or systematically. Alternatively, they may be selectively restricted



	CD56hi	CD56dim (less differentiated)	CD56dim (more differentiated)	CD56dim (adaptive-like)	Tissue resident*
Cytokine receptors	+++	+++	++	+	+/–
NKG2C	–	–	+	+++	+/–
FcεRγ	++	+++	+++	+/–	NA
NKG2D	+++	++	++	++	++
NCR	+++	++	++	+	++
CD16	+/–	++	++	+++	+/–
KIRs	–	+	++	+++	+/–
NKG2A	+++	++	+	+/–	+++
CD57	–	++	++	+++	+/–
Cytotoxic granules	+	++	+++	+++	+
Tissue-residency markers	+/–	–	–	–	+++

Box 3 | **Antigen-specific NK cell responses against viruses**

More than a decade ago, we learned of antigen-specific responses mediated by mouse natural killer (NK) cells[54,55,97]. These involved antigen-specific recall responses to haptens but also responses against viruses after prior sensitization. Intriguingly, the responses were largely confined to CXC-chemokine receptor 6 (CXCR6)-positive liver-resident NK cells[54,55]. Similarly, hepatic, but also splenic, NK cells from macaques infected with simian–human immunodeficiency virus and simian immunodeficiency virus or adenovirus vector-vaccinated animals demonstrated long-term antigen specificity in an NKG2-dependent manner[156]. Recently, such NK cell phenomena have also been reported in the human setting. First, Nikzad and colleagues showed that human NK cells, in humanized mice, exhibited vaccination-dependent, antigen-specific recall responses upon secondary challenge in vitro[49]. Interestingly, in line with findings from mouse and macaque models, these responses were also confined to hepatic CXCR6$^+$ NK cells[49]. The human liver is enriched in NK cells and contains a sizeable tissue-resident NK cell subpopulation[149]. Given this and the association between liver NK cells and antigen-specific NK cell responses from experimental models, it was no surprise that two studies in 2020 reported human liver NK cell antigen-specific responses against hepatitis A virus and hepatitis B virus following vaccination against these viruses[56,157]. The CD49a$^+$CD16$^-$ hepatic NK cells mediating these antigen-specific responses had a primed epigenetic state compared with conventional CD49a$^-$CD16$^+$ NK cells, yielding hyper-responsiveness upon IL-2 plus IL-15 stimulation[56]. However, the mechanism for antigen specificity currently remains elusive and so does the broad usefulness of these cells against other future infections.

to a specific organ due to tissue tropism (for example, the liver in the case of hepatitis virus infections). From considerable work in mouse experimental models, it is known that recruitment of circulating NK cells to the site of infection is important for the early inflammatory response and viral containment[44–47]. Chemokine–chemokine receptor interactions are necessary for mouse NK cell homing to tissues early in infection, and certain combinations come into action depending on the affected organ[45–47]. In a similar way, circulating NK cells migrate into lymph nodes in a CXCR5-dependent manner in macaques and help to control simian immunodeficiency virus (SIV) infection[48]. In humans, however, there is a relative scarcity of studies assessing NK cell homing to tissues during acute viral infections because of the inherent challenges in performing such studies and lack of easy access to infected tissue samples. Nevertheless, in acute DENV infection, responding peripheral blood NK cells show a distinct chemokine receptor imprint, including high expression of the skin-homing marker cutaneous lymphocyte antigen. Because of this, high numbers of NK cells are present in the skin of patients early after infection[22]. Similarly, skin challenge with varicella zoster virus antigens in previously vaccinated individuals led to rapid recruitment of NK cells to the site of challenge[49]. However, studies in humans are warranted where NK cell homing to tissues during acute infections can be studied.

Tissue-resident NK cells in infection. Besides homing of NK cells to tissues in response to acute viral infections, we have in recent years learned that most peripheral organs contain tissue-resident CD56^{bright} NK cells (in humans) and/or group 1 innate lymphoid cells (ILC1s) (in mice) in the steady state (reviewed in REF.[10]) (BOX 2). These cells are often strategically positioned at the site of viral infection in organs, such as the liver, female genital tract, salivary glands, kidneys and intestine, poised to respond rapidly[10,50]. Indeed, in mouse models it has been shown that tissue-resident ILC1s confer early host protection[51]. Their human counterpart is most likely tissue-resident CD56^{bright} NK cells (hereafter referred to as tissue-resident NK cells), given their functional similarities, enrichment in tissues and shared expression of tissue-residency markers (BOX 2). Since tissue-resident NK cells resemble circulating CD56^{bright} NK cells, cytokine and chemokine production is presumably the main function exhibited by these cells in early stages of infection (FIG. 1). Tissue-resident NK cells can also respond with local proliferation in acute and chronic viral infection[52]. Tissue-resident NK cells have also been shown to more directly influence the magnitude of T cell responses via expression of the inhibitory ligand PDL1, leading to viral persistence but more limited immunopathology after lymphocytic choriomeningitis virus infection[52]. In humans, a similar regulation of antiviral T cell immunity has been shown for liver-resident NK cells in chronic hepatitis B virus (HBV) infection[53]. However, compared with what has been reported for conventional NK cell responses to viral infections, much less is known regarding the role of tissue-resident NK cell responses. In this regard, it is striking to note that antigen-specific NK cell responses to vaccination and/or infection appear to be largely confined to tissue-resident NK cells in both humans and mice[49,54–56] (BOX 3). Future work should aim at disentangling the relative contributions of migrating and tissue-resident NK cells in antiviral responses occurring in peripheral organs.

NK cells in chronic viral infections

NK cells adapt to chronic viral infection in ways that are only partially understood. These adaptations likely benefit viral control over time. Impairment of the ability to handle chronic viral infections, such as herpesvirus infections, is exemplified by the not seldom lethal consequences of severe inborn deficiencies affecting NK cell development and/or function.

Hepatitis C virus infection. Although hepatitis C virus (HCV) belongs to the family *Flaviviridae*, it only rarely causes acute symptomatic disease. Instead, HCV is successful in evading the initial immune response and establishing chronic infection. There is strong evidence suggesting that NK cells contribute to HCV infection outcome, as the combination of *KIR2DL3* with homozygosity for the killer cell immunoglobulin-like receptor (KIR) ligand gene *HLA-C1* has been associated with significantly elevated viral clearance[57]. Furthermore, certain NK cell phenotypes are linked to reduced acquisition of chronic infection in exposed individuals as well as to the outcome of acute HCV infection[58,59]. Yet once the virus persists and the infection becomes chronic, the rate of spontaneous clearance is low. In this phase, the immune response against the virus is hampered at several levels. T cells from patients with chronic HCV infection display reduced proliferation and cytokine production[60], along with upregulation of inhibitory receptors[61,62], a combination of features referred to as 'exhaustion'. From the lymphocytic choriomeningitis virus infection model, it is known that NK cells can contribute to sustaining the chronic infection by targeting activated CD4$^+$

Killer cell immunoglobulin-like receptor
(KIR). A family of highly polymorphic activating and inhibitory receptors that serve as key regulators of human natural killer cell function.

HLA-E
Non-classical MHC class I molecule characterized by a limited polymorphism that normally binds a restricted subset of peptides derived from signal peptides.

T cells, which in turn leads to CD8$^+$ T cell exhaustion[63], and that NK cell depletion promotes antiviral CD8$^+$ T cell immunity[64]. A similar mechanism has also been described in chronic HBV infection, in which NK cells mediate depletion of HBV-specific T cells[53].

In chronic HCV infection, NK cells display an altered phenotype and functionality. Yet the impact of chronic infection on NK cells appears to be, at least in part, unique to each individual. In assessment of the expression of activating receptors, both elevated and reduced frequencies have been reported[59,65,66]. Along the same lines, also for NK cell degranulation both reduced levels[65,67] and elevated levels[68,69] have been reported. By contrast, a consistent effect of chronic HCV infection on NK cells appears to be reduced production of proinflammatory cytokines[68–70]. Thus, although the NK cell compartment is indeed affected in chronic HCV infection, certain aspects of this appear to rely on other factors, such as the duration of infection or the underlying state of liver disease. This effect of chronic HCV infection on the NK cell compartment is likely to be caused by a plethora of mechanisms, but it has been shown that the direct interaction of NK cells with HCV-infected cells leads to reduced expression of NKG2D and decreased functionality[71]. In line with this, NS5A-mediated production of transforming growth factor-β could also be linked to reduction of NKG2D expression[67]. In recent years, the development of direct-acting antivirals has revolutionized HCV infection therapy, as these drugs lead to successful viral clearance in nearly all patients, with negligible side effects. This has also made it possible to assess the capacity for reinvigoration of NK cell function after rapid clearance of a chronic infection (BOX 4).

HIV-1 infection. The effector capacity of NK cells includes cytotoxic elimination of HIV-1-infected target cells, as well as secretion of IFNγ, TNF and CCL4, influencing antiviral responses and limiting viral spread[72,73]. Notably, however, a differential role for cytokines, including TNF and IFNγ, has been observed in chronic states of the infection[74,75]. NK cells also produce β-chemokines, CCL3, CCL4 and CCL5, which function as natural ligands of CCR5, hampering HIV infectivity of target cells[76]. Additionally, population-level genetic associations between NK cell receptor expression and HIV-1 infection outcome and evolution have clearly revealed the impact of NK cells on HIV-1 disease progression. In HIV, *KIR–HLA* combinations have been associated with the pace of disease progression[77] and protection against disease acquisition[78]. In detail, *HLA-B Bw4-80I* in combination with *KIR3DL1*h* but also with *KIR3DS1* could be linked to improved viral control[77,79]. The mechanisms conferring this protection may include both NK cell education through inhibitory receptor ligation[80] and the direct interaction of KIRs with HIV-1-derived peptide motifs presented on HLA molecules[81,82]. Indeed, for KIR3DS1$^+$ NK cells, an *HLA-B Bw4-80I*-dependent suppression of viral replication has been shown[83], further supporting a direct role for NK cells in mediating the observed protective effect. HIV-1-mediated downregulation of HLA molecules, which prevents infected cells from lysis by CD8$^+$ T cells[84], may also facilitate NK cell recognition of infected cells. However, this is limited by preservation of expression of HLA-C and HLA-E[85]. HIV-1 infection has also been shown to trigger the upregulation of ligands for NKG2D and other activating receptors[86]. However, HIV-1 has enabled mechanisms to limit the recognition of these ligands through the expression of viral accessory proteins[87,88]. Furthermore, responses characterized by coordinated antibody function, including NK cell-mediated ADCC, are associated with viral controller phenotypes[89].

Like for many other viral infections, changes in NK cell receptor repertoire and functionality are observed during HIV-1 infection. For example, treatment-naive HIV-1-infected individuals have a significant loss of CCR7$^+$CD56^{bright} NK cells, and this change has been associated with higher viral loads[90]. Loss of the CD56^{dim} NK cell subset during HIV-1 infection has also been observed[90]. Animal models of SIV infection have shown accumulation of CD56$^-$CD16$^+$ NK cells in lymph nodes[91]. Since HIV-1 infection is often subjected to pharmacological intervention, the effects of therapies on NK cells have been studied. Initiation of antiretroviral therapy has been associated with alterations in the CD56^{bright} and CD56^{dim} NK cell populations, with the former increasing after therapy[92].

Adaptive-like NK cell expansions. Studies of human peripheral blood NK cells have revealed receptor expression-associated changes throughout life[93] that have been linked to an individual's infection history[94]. Some of these changes are evident as the appearance of adaptive-like NK cell expansions (reviewed in REF.[95]) that have been extensively studied in the mouse cytomegalovirus (MCMV) infection model[96]. In this infection, Ly49H$^+$ NK cells that specifically recognized the MCMV-encoded glycoprotein m157 were expanded

Box 4 | **NK cell reinvigoration after resolution of chronic viral infection**

Since chronic viral infections are cleared only very rarely, it is seldom possible to study immune system reinvigoration after removal of the chronic insult. For instance, antiviral treatment of HIV-1 infection can successfully suppress viral replication but not permanently remove the virus and/or infection. In this regard, the introduction of treatment with direct-acting antivirals for hepatitis C virus (HCV) has presented a unique opportunity for immunologists to address immune system restoration upon pathogen removal. While the viral loads of HCV drop rapidly upon treatment, immune functions appear to be restored only partially. For example, the proliferative capacity of HCV-specific CD8$^+$ T cells is restored[158] but mitochondrial alterations persist over time[159]. Along the same lines, the levels of many, but not all, soluble inflammatory serum proteins that are altered in chronic HCV are normalized upon viral clearance[160]. Furthermore, the unconventional mucosa-associated invariant T cell subset exhibits phenotypic and functional alterations long after viral clearance[161]. For natural killer (NK) cells, partial reinvigoration upon viral clearance has been described, although certain NK cell features appear to be affected for a prolonged period. In more detail, a reversal of NK cell activation[69] and a rapid restoration of signal transducer and activator of transcription 1 (STAT1) expression and associated type I interferon responsiveness have been described[162]. On the other hand, the diversity of the NK cell population appears not to be restored after viral clearance[163]. This might be explained by epigenetic changes caused by chronic infection that are long-lasting in nature. Indeed, in human cytomegalovirus infection, it has been shown that adaptive-like NK cell expansions display epigenetic modifications[108] and that chronic antigen stimulation is a driver for such modifications and for related functional alterations[164]. It is thus plausible that such epigenetic modifications also occur in the context of chronic HCV infection.

on primary infection[96]. These NK cells were shown to confer protection against MCMV following rechallenge with the virus[97]. Adaptive-like NK cell expansions have also been identified in humans. The very first indications of adaptive-like NK cells came from observations on the expansion and persistence of $CD94^{+}NKG2C^{+}$ NK cells in humans infected with human cytomegalovirus (HCMV)[98]. Adaptive-like NK cell expansions have also been observed following diverse viral infections in humans, including hantavirus infection[99], chikungunya virus infection[100] and HIV-1 infection[101]. Notably, all these responses correlated with HCMV co-infection, suggesting that subclinical reactivation of HCMV could be one mechanism underlying the observed responses. By contrast, several other infections, including recurrent herpes simplex virus 2 infection[102] and acute Epstein–Barr virus (EBV) infection[103], have not been associated with an expansion of $NKG2C^{+}$ NK cells. Furthermore, adaptive-like NK cell expansions observed in individuals infected with HBV, HCV and/or hepatitis delta virus were shown not to be linked to the chronic hepatitis infections per se but rather to be consequences of an underlying HCMV infection[104]. Distinct from adaptive-like NK cell expansions characterized by a reshaping of the NK cell repertoire towards certain subpopulations, additional animal models of herpes simplex virus, influenza virus and SIV infection and more recently also work in the human setting assessing vaccination responses have revealed the existence of antigen-specific NK cell responses (BOX 3).

In more in-depth recent work in humans, several characteristics of adaptive-like NK cells have been identified, including clonal expansion, persistence and recall responses[105,106]. Furthermore, these cells are characterized by low expression of the transcription factor zinc-finger protein PLZF (also known as ZBTB16), silencing of intracellular signalling molecules and commonly also expression of CD57 (REFS[107,108]). Human adaptive-like NK cell expansion depends on expression of the NKG2C ligand HLA-E[109]. Adaptive-like $NKG2C^{+}$ NK cells differentially recognize distinct HCMV strains encoding variable UL40 peptides that, in combination with proinflammatory signals, control expansion and differentiation of adaptive-like $NKG2C^{+}$ NK cells[110]. Interestingly, HCMV-seropositive individuals possessing a homozygous null allele of *KLRC2* (encoding NKG2C) remain healthy. In these individuals, the adaptive-like NK cell expansions instead expressed elevated levels of CD2, which synergized with CD16 to activate NK cells in HCMV infection[111]. It is not unlikely that there are additional environmental factors that similarly shape NK cell phenotype and function that are even less well understood. Finally, since adaptive-like NK cell expansions decrease NK cell repertoire diversity, the long-term consequences of this for the individual remain elusive.

$CD56^{-}$ NK cells in chronic infections. A particular hallmark of chronic viral infections such as HIV-1 and HCV infections is the expansion of a subset of $CD56^{-}$ NK cells (reviewed in REF.[112]). More recently, $CD56^{-}$ NK cells have also been observed to be expanded in HCMV-seropositive and EBV-seropositive elderly individuals[113]. In healthy individuals, the $CD56^{-}$ NK cell subset encompasses only a small proportion of the NK cell compartment in peripheral blood[114]. In chronic infections, however, it may constitute up to half of all peripheral NK cells, largely at the expense of the $CD56^{dim}$ NK cell subset[115–117]. The $CD56^{-}$ NK cell population displays an exhausted phenotype, with reduced levels of perforin, granzyme B, IFNγ and TNF, and lower cellular cytotoxicity than $CD56^{dim}$ NK cells[117,118]. It has remained unclear to what extent these expanded $CD56^{-}$ NK cells represent a similar or distinct phenotype as compared with the $CD56^{bright}$ or $CD56^{dim}$ NK cell populations in healthy individuals. However, more recently, an unbiased surface receptor screen and a global proteome analysis were performed to obtain detailed molecular and phenotypic information of the $CD56^{-}$ NK cell subset[119]. It was revealed that $CD56^{-}$ NK cells have a surface proteome and a total proteome related to, but not identical to, those of $CD56^{dim}$ NK cells. Functional investigations in that study examining degranulation, cytotoxicity and IFNγ production of $CD56^{-}$ NK cells from healthy individuals largely confirmed an, at most, moderate responsiveness of these cells[119].

NK cell deficiencies and infections

Studies of patients with more than 400 different primary immunodeficiency diseases (PIDs) have provided key insights into host control of viral infections. NK cells are affected in more than 50 PIDs. At present, at least seven PIDs have an abnormality specifically affecting NK cells (reviewed in REF.[120]) (FIG. 2). These specific NK cell deficiency disorders include mutations affecting total NK cell numbers, NK cell subsets and/or NK cell functions[120]. The genes affected in these seven NK cell deficiencies are *GATA2* (REF.[121]), *MCM4* (REF.[122]), *RTEL1* (REF.[123]), *GINS1* (REF.[124]), *IRF8* (REF.[125]), *MCM10* (REF.[126]) and *FCGR3A*[127]. The first six genes affect NK cell development or maturation, with resulting low total NK cell numbers and specific defects affecting NK cell subsets. *FCGR3A* gene mutations affect NK cell function despite normal numbers of NK cells[120]. This is due to a homozygous mutation in *FCGR3A* (which encodes CD16)[127,128]. Intriguingly, despite CD16 functioning as an IgG Fc receptor, patients with mutations in *FCGR3A* have impaired natural cytotoxicity but intact ADCC function[128]. The clinical presentation of human NK cell deficiency varies from patient to patient; however, hallmark viral infections affecting these individuals include herpesvirus infections such as HCMV, EBV, herpes simplex virus and varicella zoster virus infections[120,126] (FIG. 2). These viruses cause disease in almost 60% of reported NK cell deficiency cases[120]. On the basis of these findings, one may argue that host defence against herpesvirus infections might be one of the most important functions of NK cells in the context of antiviral immunity. In addition, some other viral infections have been reported in patients with NK cell deficiency, including human papillomavirus infection and some respiratory infections[120]. Interestingly, these observations suggest an important role for NK cells in keeping these, often latent, infections under control. Notably, of patients with reported NK cell deficiency, almost half

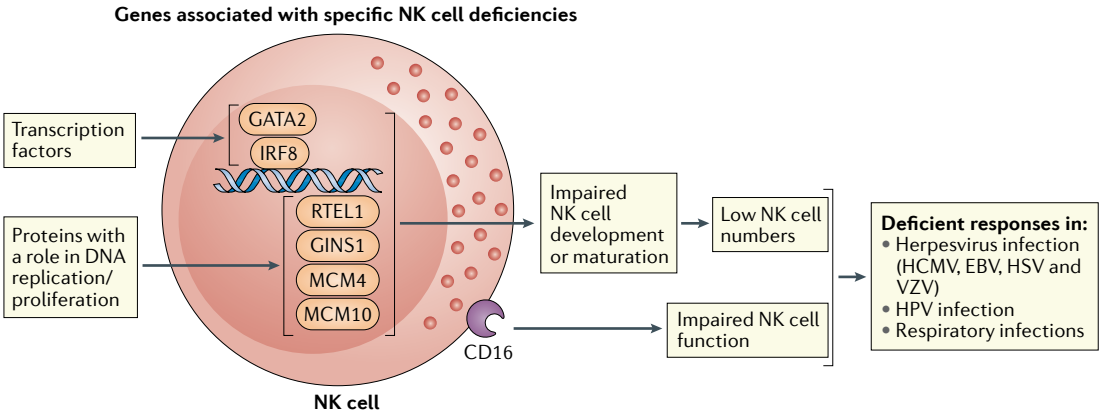


Fig. 2 | **NK cell deficiencies and viral infections.** Different genetic mutations have been linked to specific natural killer (NK) cell deficiencies. Affected genes encode either intranuclear proteins (GATA2, IRF8, RTEL1, GINS1, MCM4 and MCM10) or the surface-expressed Fc receptor for IgG CD16 (encoded by *FCGR3A*). Defects in the intranuclear proteins lead to disturbed NK cell development and/or differentiation. The mutation affecting CD16 causes altered NK cell functionality. A common denominator for NK cell deficiencies is an inappropriate response to viral infections, as evidenced by severe and/or recurrent herpesvirus infections (human cytomegalovirus (HCMV), Epstein–Barr virus (EBV), herpes simplex virus (HSV) and varicella zoster virus (VZV)), as well as human papillomavirus virus (HPV) infection and respiratory infections.

have been reported to have died prematurely[120], underscoring the severity of these diseases. Corroborating these findings, in studies of patients with a *GATA2* deficiency, the frequencies of NK cells were directly associated with increasing complications of disease[129].

NK cells in COVID-19

In late 2019, a new zoonotic viral pathogen, SARS-CoV-2, emerged and rapidly spread throughout the world, causing more than 60 million infections and 1.5 million deaths during the first year of the pandemic[130]. SARS-CoV-2 infection can cause COVID-19, a respiratory and vascular disease which, in severe cases, can lead to acute respiratory distress syndrome, multi-organ failure and death[130,131]. Although the disease pathogenesis is not yet completely understood, a misdirected and hyperactivated immune system is thought to contribute to severe COVID-19, with a likely contribution from NK cells.

NK cell response to SARS-CoV-2. NK cells are early responders to acute SARS-CoV-2 infection, with recruitment of $CD56^{bright}$ and $CD56^{dim}$ NK cells from the circulation to the lungs, leading to reduced numbers of circulating NK cells in acute infection[33,132–135] (FIG. 3). A gene module for chemotaxis is induced in lung NK cells[33,135], and lungs of patients with COVID-19 contain elevated levels of chemokines such as CCL3, CCL3L1, CCL4, CXCL9, CXCL10 and CXCL11 (REFS[135,136]). Although further work is needed in the area, this chemokine production suggests that CXCR3 and CCR5 are important for homing of NK cells to the lungs in acute SARS-CoV-2 infection[137]. The early depletion of NK cells from the circulation and their redistribution to the site of infection is in line with what has been observed in other severe acute infections, such as acute hantavirus infection[99]. Furthermore, NK cells display an activated and cycling phenotype in acute SARS-CoV-2 infection at both the protein level and the transcriptomic level, with upregulation of Ki67, CD69, HLA-DR and CD38 (REFS[33,132,134]). In the $CD56^{dim}$ NK cell compartment, less differentiated $NKG2A^{+}CD62L^{+}CD57^{-}KIR^{-}$ cells were the main responding cells, suggesting a cytokine-driven mechanism of activation[33] (BOX 1; FIG. 1). Paralleling the activation of NK cells, regulatory programmes appear to be initiated, as evidenced by the upregulation of inhibitory checkpoint receptors such as LAG3, TIGIT and TIM3 (REFS[33,132]) (FIG. 3). This could possibly explain why peripheral blood NK cell function has been reported to be blunted in acute SARS-CoV-2 infection[133,138].

Role of NK cells in COVID-19. Although NK cells appear to be robustly activated and home to the lungs in acute SARS-CoV-2 infection, from the currently available studies, the level of NK cell activation (induction of CD69 and HLA-DR expression) or proliferation is not associated strongly with COVID-19 severity. Instead, Maucourant and colleagues reported that severe/critical COVID-19 was associated with the appearance of adaptive-like NK cell expansions[33] (FIG. 3). These expansions where characterized by high NKG2C and CD57 expression as well as by narrow KIR profiles[33]. Since the first report, similar findings have been reported in two additional studies[133,139]. It currently remains unclear whether the increased frequency of adaptive-like NK cells in severe COVID-19 is because of a direct effect of SARS-CoV-2 or whether this is a bystander phenomenon driven by HCMV[33]. Indeed, a history of HCMV infection appears more common in patients with severe COVID-19 (REF.[140]), and some evidence of local HCMV reactivation in the lungs of patients with COVID-19 receiving ventilator treatment has been reported[141]. On the other hand, both immune and parenchymal cells in the lungs of patients with COVID-19 display elevated HLA-E expression[33], and it has been suggested that the SARS-CoV-2 spike protein can induce surface HLA-E expression by lung epithelial cells[142]. However, it

remains to be determined whether upregulated HLA-E will stimulate NK cells via NKG2C or rather inhibit the NKG2A-expressing NK cells.

Future research should also address whether expansion of adaptive-like NK cells is needed to unleash the full antiviral capacity of NK cells against SARS-CoV-2 infection or whether these cells are instead contributing to COVID-19 pathogenesis. In this regard, it is interesting to note that a recent artificial intelligence-guided big-data approach identified a 'severe viral pandemic core gene signature' that highlighted IL-15–IL-15RA and NK cell senescence as important determinants for severe or fatal COVID-19 (REF.[143]). Corroborating this, it was recently suggested that IL-15 could be linked to NK cell dysfunction in COVID-19 and that this occurs late after symptom onset and only in the most severely ill patients[144]. Thus, these studies support a model in which misdirected and/or exhausted NK cell responses, rather than hyperactivated NK cells, contribute to severe COVID-19. Future work should explore the contribution of myeloid-derived suppressor cells and immature neutrophils[145] in relation to NK cell dysfunction in severe COVID-19 (FIG. 3).

Concluding remarks and outlook

As is evident from studies reviewed herein, it is clear that NK cells can mount vigorous responses to several acute viral infections, including acute flavivirus and influenza virus infections as discussed here. In many situations, this triggers homing of circulating NK cells to affected tissues.

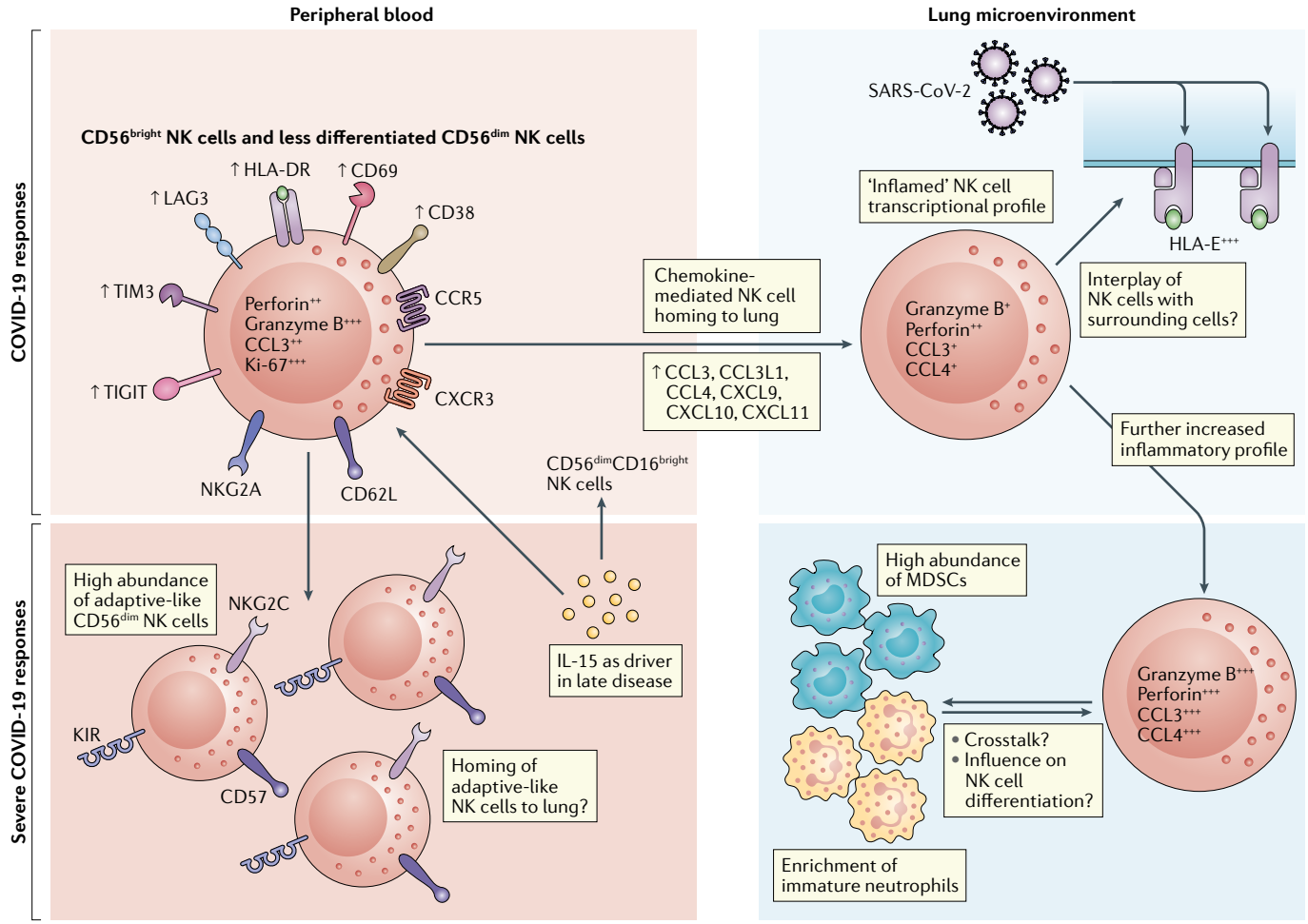


Fig. 3 | **NK cells in COVID-19.** In the circulation, natural killer (NK) cells respond strongly to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and display an altered phenotype, including elevated expression of activation markers (HLA-DR, CD69 and CD38), inhibitory molecules (TIM3, LAG3 and possibly PD1) and tissue-homing markers (CCR5, CXCR3 and CD62L). Furthermore, circulating NK cells are highly proliferative and upregulate perforin and granzyme B expression. This response is primarily confined to less differentiated NK cells, suggestive of a cytokine-driven response. NK cells likely home to the lungs, where they exhibit an inflamed transcriptional signature. In severe coronavirus disease 2019 (COVID-19), adaptive-like NK cells are found at higher frequencies in the circulation, but it remains unclear whether these cells home to the lungs and interact with infected epithelia that show increased expression of HLA-E, a ligand for the activating receptor NKG2C. Furthermore, the transcriptional profile of NK cells in the lung microenvironment of patients with severe COVID-19 is even further skewed towards inflammation. This lung microenvironment also contains high numbers of myeloid-derived suppressor cells (MDSCs) and immature neutrophils. However, details on how NK cells might interact with these cells remain elusive. Additional outstanding questions related to NK cells in COVID-19 are highlighted in the figure. CCL, CC-chemokine ligand; CCR, CC-chemokine receptor; CXCL, CXC-chemokine ligand; CXCR, CXC-chemokine receptor; KIR, killer cell immunoglobulin-like receptor; LAG3, lymphocyte activation gene 3; TIGIT, T cell immunoreceptor with immunoglobulin and ITIM domains; TIM3, T cell immunoglobulin mucin receptor 3.

In parallel, less well-characterized tissue-resident NK cells may also become activated. Critical in all host responses to acute viral infections is the balance between acute antiviral mechanisms and responses contributing to tissue damage and immunopathology. Clearly, more knowledge is needed with respect to direct NK cell-mediated interactions with viral infected cells and the consequences of possible viral clearance by such interactions. NK cells adapt to chronic viral infections in ways that are only partially understood. These adaptations likely benefit viral control over time, although the detailed mechanisms are far from completely understood. Impairment of the ability to handle chronic viral infections, such as herpesvirus infections, is exemplified by the lethal consequences of severe inborn deficiencies affecting NK cell development and/or function. As discussed, at least seven PIDs have an abnormality specifically affecting NK cells. With the recent COVID-19 pandemic, NK cells have come into the spotlight in this context as well. Although the disease pathogenesis is still being worked out, a misdirected and hyperactivated immune system is thought to contribute to severe COVID-19, with a likely significant contribution from NK cells (FIG. 3).

While many insights with respect to a possible role for NK cells in viral infections have been revealed, from studies in mouse models to studies in humans, there are several outstanding questions and unresolved issues. What is the nature of NK cell viral antigen specificity as observed in, for example, hepatitis A virus and HBV vaccination (BOX 3)? Furthermore, regarding antigen-specific NK cell responses, what are specific surface receptor-mediated responses versus what might be novel forms of antigen-specificity 'dictated' by changes at an epigenetic level (BOX 3)? On the same note, what drives adaptive-like expansions of NK cell populations? What is the function of these cells in the context of viral infection? How are NK cells driven towards sites of infection and what is the role of responses from tissue-resident NK cells in the context of acute infections? Is there a division of labour between infiltrating NK cells and tissue-resident NK cells? What distinguishes functional antiviral NK cell responses from disease-causing responses? This is a question of relevance with regard to both acute responses, like in the SARS-CoV-2 situation (FIG. 3), and chronic infections, such as HIV-1 and HCV infections. With regard to vaccination, a presently hot topic, what is the role of NK cells in the generation of efficient vaccine responses? Finally, while this has been discussed for years, is there a role for NK cells in settings of adoptive immunotherapy for viral diseases such as what we are currently witnessing in the context of human malignant diseases?

In summary, although we have gained a great deal of knowledge of NK cells in the context of human viral infections over the past 10 years, much more remains to be learned.

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