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Natural Killer Cell Responses to Infections in Early Life

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Abstract
Natural killer (NK) cells are an important component of innate immune responses to infectious diseases. They mediate protection by being able to rapidly lyse infected cells and produce cytokines (primarily interferon-γ) that shape innate and adaptive immune responses. This review summarizes current knowledge on the phenotype and functional abilities of NK cells from healthy newborns/infants and on NK cell responses against viral, bacterial and protozoan infections in early life. Interestingly, NK cell blood counts are higher in newborns than in adults but they do not display striking differences in phenotype, except for an increased frequency of expression of the inhibitory CD94/NKG2A receptor. They display some inherent functional defects, mainly a lower cytolytic capacity that may contribute to the immaturity of the neonatal immune system. Changes in circulating levels of NK cells observed during pediatric infections and the ability of NK cells from newborns and children to produce interferon-γ at the encounter with pathogens indicate that NK cells participate in the immune response to infectious diseases in early life. Unfortunately, information is currently insufficient to assess whether these NK cell responses really contribute to control infections, either vertically transmitted or acquired in infancy.

Innate immune responses are characterized by their rapidity and are important in limiting the spreading of invading pathogens before adaptive immune response builds up. Natural killer (NK) cells defined as $CD3^-$ $CD56^+$ lymphocytes in humans are innate lymphocytes identified in the 1970s through their capacity to kill tumor cell lines in vitro [1]. Since then, innate lymphocytes also encompass particular subsets of B and T lymphocytes (B1-B cells, invariant NK T cells and a subset of γδ T cells) expressing germline-encoded antigen receptors [2]. NK cells are rapidly activated by cytokines and contact-dependent signals provided by dendritic cells, monocyte/macrophages and $CD4^+$ T cells having encountered pathogens [3, 4]. Constitutive expression of a complex repertoire of surface receptors enables them to directly sense infected or transformed cells. Activated NK cells mediate protection against pathogens through secretion of cytokines, primarily interferon (IFN)-γ, that shape in-

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nate and adaptive immune responses, as well as destruction of infected cells [5].

As compared to adults, newborns and children present some immunological immaturity, particularly in mounting efficient type 1 immune responses against intracellular pathogens. Several mechanisms account for such deficiency, including deficient pattern recognition receptor signaling, deficient production of interleukin (IL)-12p35 by antigen-presenting cells and of IFN-γ by $CD4^+$ T cells [6–8], as well as impaired NK cell functions. This review aims to specifically summarize current knowledge on NK cell responses to infections in early life. In the first part, we will compare the phenotype and the functional abilities of neonatal and adult NK cells, before describing in the second part NK cell responses against viral, bacterial and protozoan infections in early life.

Characterization of NK Cells in Early Life

Blood Levels of NK Cells

Levels of circulating NK cells gradually increase with gestational age. Between 25 and 30 weeks of gestation, their count represents one third to half of that found in umbilical cord blood (CB) of full-term newborns [9]. Absolute counts of NK cells in CB of term neonates average 500/mm^3, with important variations between individuals (reported levels ranging from 20 to 1,600 NK cells/mm^3) [9–12]. CB contains the highest level of NK cells, likely in relation to labor-related stress. NK cell levels then rapidly decrease by 2–3 times in the blood of newborns few days after delivery [11–13] and continue to progressively decline during childhood [11], reaching adult levels (mean of 200 NK cells/mm^3) around 5 years of age [11, 12].

$CD56^{bright}CD16^{dim/neg}$ and $CD56^{dim}CD16^+$ NK cells define two functionally distinct mature NK cell subsets. The former secretes high levels of cytokines, is poorly cytotoxic and preferentially homes into lymph nodes, while the latter appears to be mainly cytotoxic effector cells that infiltrate inflamed tissues. The majority (about 90%) of adult blood NK cells are $CD56^{dim}$, while $CD56^{bright}$ NK cells make up 75–90 and 50% of resident NK cells in lymph nodes and the spleen, respectively. They are also numerous in the decidua during gestation [5, 14]. The proportions of circulating $CD56^{bright}$ and $CD56^{dim}$ subsets are rather stable over time between birth and adult life, though $CD56^{bright}$ NK cell numbers were reported, by some authors including our group, to be slightly higher in newborns [12, 15, 16].

Neonatal Repertoire of NK Receptors and Other Surface Molecules

NK cell function is tightly regulated by activating and inhibitory signals that are delivered by a diverse array of cell surface receptors [5]. These receptors recognize self-ligands or microbial molecules expressed on infected cells. The expression of some of them has been studied on CB NK cells. Results are summarized in table 1. The main features to be put forward are the following. First, the balance between the inhibitory receptor CD94/NK cell group 2 (NKG2)A and the activating receptor CD94/NKG2C (both binding to the same ubiquitously expressed human leukocyte antigen-E molecule) [17] is much more deviated towards inhibition in neonates than in adults. This is reminiscent of the phenotype of decidual NK cells which are $CD94/NKG2A^{bright}$ and killer cell immunoglobulin-like receptor $(KIR)^-$, a phenotype associated with NK cell function orientated towards cytokine secretion rather than towards cytotoxicity [14]. Expression of the other activating and inhibitory receptors is variable compared to adults. Since the combination of expression of these receptors may vary between cells, it is not possible to predict the net effect and compare neonatal and adult cells. We can also note that leukocyte immunoglobulin-like receptor (LIR)-1, which binds to classical major histocompatibility complex class I with a broader recognition pattern than KIRs, is very low in CB; adult levels will be reached around 5 years of age [12, 15].

CD244 and CD2, belonging to the CD2 family of receptors and binding to CD48 and CD58, respectively, alongside mediating adhesion with target cell, also give costimulatory signals [18]. CD244 expression on NK cells is similar in newborns and adults [12] while the proportion of $CD2^+$ NK cells is slightly higher in CB [19]. Finally, CB NK cells also express adult levels of CD16, the activating FcγRIII receptor which recognizes IgG and mediates antibody-dependent cellular cytotoxicity (ADCC) [12, 20].

Cytotoxic Capacity

The natural killer cytotoxic (NKC) capacity of CB NK cells, i.e. their capacity to spontaneously and rapidly lyse cognate targets without undergoing previous gene transcription and differentiation, has been extensively studied since the 1980s. Most reports converge to show that NKC capacity is strongly reduced in early life as compared to adults, although some newborns display adult-like cytotoxic function [21]. Indeed, even if NKC was detected in liver cells as early as 9 weeks of gestation [21], it remained at least 3-fold lower in newborns at term than

Table 1. Surface expression of receptors and adhesins on circulating neonatal and adult NK cells

NKR or surface marker	Function	Ligand[1]	Expression level on resting NK cells[2]			
			newborn	adult	newborn vs. adult	reference
KIRs[3]		classical MHC class I (HLA-A, B, C)				
2DL1/DS1 (CD158a/h)	inhibitory or activating R		+	+/++	↓/≈	12, 19
2DL2-3/DS2 (CD158b/j)	inhibitory or activating R		+	+/++	↓/≈	12, 19
3DL1/DS1 (CD158e 1/2)	inhibitory or activating R		+f/+	+	↓	12
LIRs						
LIR-1 (CD85j, ILT-2)	inhibitory R	MHC class I (HLA-A, B, C and E) *UL18 (hCMV)*	+f/+	++/+++	↓	12, 15
C-type lectin receptors						
CD94/NKG2A[4]	inhibitory R	non-classical MHC class I (HLA-E)	++++	+/++	↑↑	12, 15
CD94/NKG2C[4]	activating R	non-classical MHC class I (HLA-E)	+f/+	+f/+	≈	12, 15
NKG2D	activating R	MHC class-I-like molecules (ULBP 1 to 6, MIC A and B)	++++	+++++	↓	12, 15
LAIR-1	inhibitory R	collagen	+++++	+++++	≈	12
CD161	inhibitory R	LLT-1 (CLEC2D)	+++++	+++++	↑	70
NCRs						
NKp30 (NCR3, CD337)	activating R	B7-H6 BAT3 (HLA-B associated transcript 3) *pp65 of hCMV*	+++++	+++++	↑	12, 15
NKp44 (NCR2, CD336)	activating R	cells in mitosis *MTb, HA (influenza virus)*	+f	+f	≈	12, 15
NKp46 (NCR1, CD335)	activating R	cells in mitosis	+++++	++++	↑	12, 15
NKp80	activating R	CLEC2B	+++++	+++++	≈	12
CD16 (FcγRIII)	activating R	complexed IgG			≈	12, 15, 19
CD2	adhesion/costimulation	CD58 (LFA-3)	+++	++/+++	≈	19
CD244 (2B4)	adhesion/costimulation	CD48	+++++	+++++	≈	12, 18
CD62L	adhesion	carbohydrate ligands on endothelium	+f/+	+/++	↓	19
CD11a (LFA-1)	adhesion	ICAM-1 (CD54), ICAM-2 (CD102)	+++++	+++++	≈	19
CD11b	adhesion	ICAM-1, fibrinogen, iC3b, *LPS, β-glucan*	+++++	+++++	≈	19
CD11c	adhesion	ICAM-1, fibrinogen, iC3b, *LPS, β-glucan*	++++	++++	≈	19
CD54 (ICAM-1)	adhesion	$\beta_2$integrins : LFA-1, LFA-2	++	+++++	↓↓	19
CD8αα	induces Fas-FasL apoptosis of NK cells	soluble MHC class I	++	+++	↓	19, 70
CD69	marker of activation retention in lymphoid organs, immunoregulation?	unknown	+f/+	+f/+	≈	12, 15
CD57	marker of terminal differentiation (oligosaccharide moiety of surface proteins)	–	+f	++/+++	↓	12, 19

MHC = Major histocompatibility complex; HLA = human leukocyte antigen; ILT-2 = immunoglobulin-like transcript 2; MIC = MHC class 1 chain-related molecule; ULBP = UL16-binding protein; LAIR = leukocyte-associated immunoglobulin-like receptor; LLT-1 = lectin-like transcript 1; NCRs = natural cytotoxicity receptors; CLEC2B and CLEC2D = C-type lectin domain family 2 members B or D; HA = hemagglutinin; ICAM = intercellular adhesion molecule; LFA = lymphocyte function-associated antigen.

[1] Microbial ligands are indicated in italics, others are self-ligands, either constitutively expressed or induced/released by stressed/infected/tumoral cells. [2] +++++ = ≥90% of NK cells; ++++ = 70–90%; +++ = 50–70%; ++ = 30–50%; + = 10–30%; +f < 10 %. [3] KIRs are characterized by 2 (KIR2D) or 3 (KIR3D) extracellular Ig domains. In addition, they have either short (S) or long (L) intracytoplasmic tails which transduce activating or inhibitory signals. [4] The proportion of NK cells expressing CD94 does not vary [19], suggesting that the NKG2 isoform is preferentially associated with variation in age.

in adults [19, 21]. Decreased number of cytoplasmic granules, poor degranulation ability (i.e. low expression of CD107c, a marker of degranulation) [12] and impaired release of lytic factors have been revealed in CB NK cells, along with variable levels of perforin and granzyme contents [19, 22]. Surface expression of Fas-ligand or tumor necrosis factor-related apoptosis-inducing ligand is comparable to adults [19]. The ability of CB NK cells to perform ADCC has been reported to be similar or reduced as compared to adult cells, although the expression of the FcγRIII (CD16) was normal [12, 20].

Adhesion between NK and target cells is a prerequisite for cytolysis to proceed. Studies have shown either normal binding of neonatal NK cells to virus-infected cells [20] or diminished binding to K562 cells as compared to adult NK cells [23]. The expression of the β_2-integrin lymphocyte function-associated antigen 1 (CD11a/CD18 heterodimer), playing a central role in tight adhesion as well as in polarization of cytolytic granules toward the immunological synapses [24] is reported to be normal on neonatal NK cells while other adhesins are diminished [19, 20]. Whether these differences could result in adhesion defects still needs to be addressed. Defective cytotoxic capacity of neonatal NK cells may also result from poor quality of signaling through NK receptors (NKRs) as it is the case for other receptors [6]. However, this has not been investigated to date.

Interestingly, cytokines able to activate NK cells, like interleukin (IL)-2, IL-12, IL-15 and IFNs, are able to rescue cytotoxic defects in vitro by increasing adhesion molecules, perforin/granzyme contents and the level of granule exocytosis, allowing to reconstitute cytotoxic capacity to adult levels if sufficient amounts of cytokines are present [12, 25, 26]. This was the case in one study where IFN-α produced during in vitro infection with herpes simplex virus restores cytotoxic capacity of CB NK cells to adult levels [27]. The magnitude of cytotoxicity restoration will depend on the amounts of activating cytokines, and sufficient levels may not be found in newborns. Indeed, the capacity to produce IL-15, IL-12 and IFN-γ in response to pathogens is dampened in early life [6, 7, 28].

IFN-γ Production

Another important function of NK cells relates to the production of cytokines which have been shown to modulate developing immune responses. Studies have mainly focused on IFN-γ production, which has recently been shown to be presynthesized and stored in cytosolic granules (different from those containing cytolytic enzymes) to be rapidly released upon activation [29]. CB NK cells do not spontaneously produce IFN-γ, while the low level of this cytokine is released in vitro by adult cells [30]. Strong stimulation with phorbol ester and ionomycin activated a similar proportion of NK cells purified from CB and adult blood to produce IFN-γ production [19], although the amount of IFN-γ finally released by neonatal cells was lower [30]. IL-12, the canonical inducer of IFN-γ, induced similar levels of IFN-γ mRNA and protein in cord and adult NK cells [30], while when combined with IL-18, it boosted a still higher release of IFN-γ by CB NK cells (by both $CD56^{bright}$ and $CD56^{dim}$ subsets) as compared to adult blood NK cells [12, 31].

NK Cell Response to Infections Acquired in Early Life

The data gathered on neonatal NK cells from healthy newborns seem to indicate that they present inherent defects more prominent at the level of their cytotoxic activity. In addition, it has been reported that soluble factors present in CB like prostaglandins and soluble human leukocyte antigen-G [32, 33], or cells such as $CD4^+CD25^{high}$ regulatory T cells more numerous in neonates, may contribute to limit NK cell functions [34, 35]. In addition, CB NK cells have been shown to be more prone to spontaneous apoptosis than their adult counterparts, despite their lower expression of Fas [36]. All these features should limit the ability of neonatal NK cells to fight infectious diseases due to intracellular pathogens. We review below what is known on NK cell responses to various infections.

Viral Infections

NK cells play a crucial role in the resolution of most viral infections, as evidenced by increased susceptibility when NK cell numbers are lacking prior to infection [37]. Humans genetically deficient in NK cells are particularly susceptible to herpesviruses [cytomegalovirus (CMV), Epstein-Barr virus (EBV), herpes simplex virus], varicella zoster virus infection, papillomaviruses, as well as to viral infections of the respiratory tract [37, 38], with severe and life-threatening recurrent infections in early life.

Severe acute respiratory infections with influenza virus or respiratory syncytial virus or severe varicella in the first years of life are associated with reduced levels of circulating NK cells [39–42]. Whether this relates to relocalization of cells in the periphery or to apoptosis is not known. However, influenza virus has recently been shown to infect NK cells and induce their apoptosis [43]. NK cell blood levels are also slightly decreased in human

immunodeficiency virus (HIV)-infected infants as compared to uninfected ones. Both NK subsets are diminished. The drop in $CD56^{bright}$, but not $CD56^{dim}$, NK cells seems to be related to a higher viral load and lower $CD4^+$ T-cell counts [44–46]. On the contrary, children undergoing active but asymptomatic CMV infection (excreting CMV DNA in urine and saliva) or chronic/latent infections with CMV or EBV do not present alterations in NK cell proportions [47, 48].

Viruses are known to induce modifications of the repertoire of NKRs. NK cell-specific triggering surface molecule (NKp)46 plays a critical function in the eradication of influenza virus [38] and its expression is increased in adults suffering from influenza [45]. At odds, its expression does not vary during severe influenza in children [41]. Pediatric CMV infection is associated, as in adults, with expansion of $CD94/NKG2C^+$ NK cells. This is particularly prominent in children with viral excretion [47]. Meanwhile, although coexpression of the inhibitory receptor LIR-1 (known to bind the viral-encoded protein UL18) increases on $CD94/NKG2C^+$ cells, the expression of CD94/NKG2A is not modified [47]. These data suggest that $CD94/NKG2C^+$ NK cells may participate in the early control of acute infection, and activating signals delivered by CD94/NKG2C may be later dampened by the acquisition of LIR-1. In contrast to what happens in CMV infection, NKG2A and some inhibitory KIRs are, or tend to be, increased in HIV-infected children [46]. This shift in the NKR repertoire, by enhancing inhibitory signals, could contribute to limit NK function and favor persistence of the virus. In line with this, NK degranulation seems to be suboptimal in HIV-infected children, as indicated by the lower expression of CD107 [46]. However, strangely, disease progression assessed by lowering of $CD4^+$ T-cell counts is associated with increased expression of activating receptors NKG2C and NKp46 [46].

An appealing role of NK cells in HIV infection concerns its potential to limit perinatal transmission of HIV from the infected mother to the newborn. NK cells have been shown to inhibit in vitro HIV replication in infected cells by producing large amounts of CC chemokine ligand (CCL)3, CCL4 and CCL5 that inhibit CC chemokine receptor-5-dependent entry of HIV into target cells [49]. Interestingly, the suppressive effect of neonatal NK cells was greater than that of adult cells [50]. Strongly supporting a protective role of NK cells in vertical transmission of HIV, a recent study shows that uninfected neonates from HIV-infected mothers display a higher proportion of NK cells that produce IFN-γ in the presence of HIV peptides than infected infants from HIV-infected mothers [51]. This is the first report of an in utero 'sensitization' of NK cells by the mother. The underlying mechanism is not known.

In adults, EBV primo infection, causing infectious mononucleosis, is accompanied by higher circulating IFN-γ levels and increased NK cell activity [52]. On the contrary, EBV-seropositive children display lower plasma IFN-γ levels than seronegative ones, which is associated with reduced ability to produce IFN-γ production by NK cells from infected infants [48]. Coinfection of these young children with CMV still decreases IFN-γ levels. The reason of the difference between adults and young children is not known. However, it might contribute to the fact that children, contrary to adults, generally have asymptomatic primary EBV infection. Indeed, symptomatic mononucleosis is related to overproduction of inflammatory cytokines [52]. Recent findings of K. Bendjela et al. [pers. commun.] show that blood NK cells from respiratory syncytial virus-infected infants also displayed a reduced capacity to produce IFN-γ, compared with controls.

Bacterial Infections

NK cells also play a role in the control of bacterial infections, as indicated by the association of recurrent bacterial otitis media and sinusitis in young children with NK cell genetic defects [37]. However, information on NK cells in children suffering from bacterial infections is scarce. NK cells of infants experiencing bacterial infection during the first year of life, due to either group B *Streptococcus, Staphylococcus aureus, Serratia* or *Escherichia coli,* present an activated phenotype [53], whereas neonates undergoing early neonatal sepsis display strongly reduced NK cell cytotoxicity as compared to healthy neonates, although the number of NK cells was not really affected [54].

The importance of NK cells in controlling *Mycobacterium tuberculosis* (MTb) infection is still debated. Data suggest that NK cells might even have a detrimental role by inhibiting protective immunity [38]. The observation that many people have repeatedly negative tuberculin skin tests and remain healthy despite extensive exposition to the risk of infection suggests that the innate immune response in some individuals controls the infection [55]. Moreover, NK cells from patients presenting active disease exhibit impaired cytotoxicity [56], supporting the involvement of NK cells in the control of disease progression. NK cells in tuberculosis (TB) are mainly activated through NKp46 and NKG2D. Their beneficial effect relies on their ability to kill MTb-infected monocytes as

well as to release IFN-γ that sustains a protective $CD8^+$ T-cell response. They also favor the development of protective Th1 response by eliminating MTb-specific expanding regulatory T cells [38, 55]. In children, particularly before 2 years of age, MTb infection progresses to severe disease more frequently and rapidly than in adults. The risk of disease progression decreases during childhood [57]. NK cell response in TB has not been studied in children, but we can speculate that the constitutively lower NKG2D expression (table 1), NK cytotoxic capacity and the ability to produce IFN-γ in early life might contribute to a higher susceptibility of infants to TB. It would also be interesting to have information on the capacity to produce IL-22 in early life, as a subset of NK cells have recently been shown to produce IL-22 that indirectly limits in vitro intracellular growth of MTb [58].

Protozoal Infections

In adults, NK cells appear to be essential in controlling infections with intracellular protozoan, including malaria, toxoplasmosis, leishmaniasis and trypanosomiasis, during the early phase of the immune response [for a review, see ref. 59]. Except for malaria in which NK-mediated killing of infected red blood cells likely significantly contributes to protection, NK cell effector function in protozoan infections is rather cytokine mediated, with a major contribution of NK-derived IFN-γ in early resistance. NK cells may be directly activated by parasite molecules like the lipophosphoglycan of leishmania interacting with Toll-like receptor 2 [60] or by yet unidentified molecules on red blood cells infected with *Plasmodium* [59, 61]. However, activation of NK cells through contact-dependent signals and cytokines released by monocytes and dendritic cells (mainly IL-12) or $CD4^+$ T cells (IL-2) seems to prevail upon NKR-mediated activation [4, 59].

Again, few data are available on the role of NK cells in early life towards protozoans. In children with clinical malaria due to *Plasmodium falciparum,* a positive correlation has been observed between the degree of parasitemia and NK cell cytotoxic capacity, while NK cells are activated to produce higher levels of granzyme A and B as well as IFN-γ [62, 63]. These data suggest that NK cells might be an essential component of innate protective immunity in early life. However, another study indicates that γδ T cells were predominant over NK cells in rapidly producing IFN-γ in response to infected red blood cells [64]. This study, performed with children 5–14 years old, does not preclude a predominant role of NK cells in neonates as IFN-γ providers, since γ9δ2 T-cell levels are strikingly less frequent at birth than after [65].

Human congenital infection can represent an interesting model of acute infection to investigate NK cells in early life. Our group has addressed the possibility that fetal NK cells play a role in the generation of the early cellular response during congenital infection. We previously described for the first time that fetuses were able to develop mature $CD8^+$ T-lymphocyte responses in response to congenital infection with *Trypanosoma cruzi,* the protozoa agent of Chagas disease in South America [66], indicating that neonatal immune defects are not absolute. To investigate the mechanisms underlying the capacity of this intracellular pathogen to overcome fetal immune immaturity, we have studied the phenotype and activity of CB NK cells in such newborns [16]. We found that they displayed a reduced proportion of circulating $CD56^{bright}$ NK cells, suggesting NK cells may have been recruited to secondary lymphoid organs. The remaining $CD56^{bright}$ NK cells exhibited a defective ability to produce IFN-γ in response to cytokines as compared with cells from uninfected newborns. In addition, $CD56^{dim}$ NK cells from congenitally infected newborns stimulated with cytokines have a decreased capacity to release granzyme B, a defect associated with a reduced surface expression of activating NKRs (NKp30, NKp46 and NKG2D), as compared with uninfected newborns, while the expression of the inhibitory receptors NKG2A and LIR-1 remained similar. Thus, NK cell responses appear to be reduced at birth after congenital transmission of *T. cruzi.* To clarify whether this impaired NK cell response is a physiological result of their previous in utero activation triggered by congenital infection, or whether live *T. cruzi* present in infected fetuses have inhibited the NK cell response (which could represent a way for the parasite to improve its survival in the host), we have studied the effect of the parasite on CB NK cells from healthy newborns in vitro. We have observed that *T. cruzi* rapidly triggers, in a dose-dependent way, IFN-γ production by both $CD56^{bright}$ and $CD56^{dim}$ subsets of NK cells (fig. 1a). IFN-γ induction is also found at the mRNA level (fig. 1b). IFN-γ release was strongly enhanced in the presence of low doses of IL-15 [16]. Activation of neonatal NK cells by the parasite is also evidenced by upregulation of surface expression of CD54 (intercellular adhesion molecule 1) and CD69, and by downregulation of CD62L. However, *T. cruzi* alone exerted only a small direct activation of NK cells, and we demonstrated the need for the presence of monocytes, suggesting that the participation of accessory cells is required [unpubl. data]. In conclusion, our work suggests that neonatal NK cells are fully functional to respond to congenital infection.

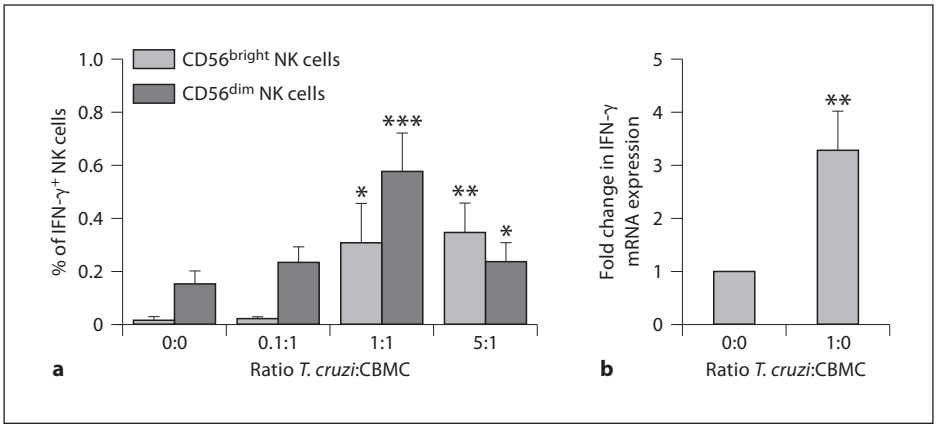

Fig. 1. IFN-γ production by neonatal NK cells in response to *T. cruzi*. CB mononuclear cells (CBMC) were incubated for 24 h with *T. cruzi* live trypomastigotes at different parasite to cell ratios. Intracellular IFN-γ was then detected by flow cytometry in $CD3^-$ $CD56^{bright}$ or $CD56^{dim}$ NK cells (**a**, n = 12 samples), and IFN-γ transcripts were measured by quantitative RT-PCR in CB mononuclear cells. IFN-γ mRNA levels were calculated using the $2^{\Delta\Delta CT}$ method, using GAPDH as calibrator and non-stimulated cells as reference (**b**, n = 7 samples). Results are expressed as the mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, statistical comparison with the absence of parasites (Wilcoxon signed-rank test).

Concluding Remarks

Though they are more numerous in blood, neonatal NK cells display some inherent qualitative defects contributing to the immaturity of the neonatal immune system. Such immaturity is probably essential to avoid deleterious overproduction of inflammatory cytokines, susceptible to provoke brain damages [67], when the newborn suddenly comes into contact with an overwhelming amount of (commensal) microorganisms during the first days of life. The cost of this immaturity in early life is an increased susceptibility to infectious diseases.

The modifications of circulating levels of NK cells observed during pediatric infections, their ability to produce IFN-γ and undergo modifications of the NKR repertoire at the encounter with pathogens argue for their engagement in early life in the immune response in infectious diseases. Nevertheless, information is currently too limited and sparse to assess to which extent these NK cell responses really contribute to the control of infections, either vertically transmitted or acquired in infancy.

To answer such questions, some main points deserve major consideration and benefit of investigations, such as (1) the real ability of NK cells from infected newborns and children to produce cytokines in response to pathogens, to lyse infected target cells (and not only K562 cells) and to home into lymph nodes or peripheral tissues, compared to that of adult NK cells from infected individuals; (2) the role of maternally transmitted antibodies in ADCC by NK cells against infectious agents, a topic poorly studied to date; (3) the role of long-term exposure to pathogens in early life (like infections with herpesviruses) in shaping the NKR repertoire, in 'arming' NK cells and in generating memory-like NK cells, concepts which have recently emerged from experimental studies [68, 69].

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