Clinical & Experimental Immunology
The Journal of Translational Immunology

Clinical and Experimental Immunology REVIEW ARTICLE doi: 10.1111/cei.13565

Immunological basis of early clearance of *Mycobacterium tuberculosis* infection: the role of natural killer cells

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Accepted for publication 7 December 2020
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Summary

Tuberculosis (TB) kills more people than any other single infectious disease globally. Despite decades of research, there is no vaccine to prevent TB transmission. Bacille Calmette–Guérin (BCG) vaccine, developed a century ago, is effective against childhood (disseminated and miliary) TB. However, its protective efficacy against pulmonary TB varies from 0 to 80% in different populations. One of the main reasons for the lack of an effective vaccine against TB is the lack of complete understanding about correlates of protective immunity on which to base vaccine design and development. However, some household contacts who are extensively exposed to *Mtb* infection remain persistently negative to tuberculin skin test and interferon-gamma assay. These individuals, called 'resisters', clear *Mtb* infection early before the development of acquired immunity. The immunological basis of early *Mtb* clearance is yet to be established; however, innate lymphocytes such as monocytes/macrophages, dendritic cells, neutrophils and natural killer cells, and innate-like T cells such as mucosal-associated invariant T cells, invariant natural killer (NK) T cells and gamma-delta (γδ) T cells, have been implicated in this early protection. In recent years, NK cells have attracted increasing attention because of their role in controlling *Mtb* infection. Emerging data from animal and epidemiological studies indicate that NK cells play a significant role in the fight against *Mtb*. NK cells express various surface markers to recognize and kill both *Mtb* and *Mtb*-infected cells. This review presents recent advances in our understanding of NK cells in the fight against *Mtb* early during infection, with emphasis on cohort studies.

Keywords: cytokines, early clearance, immunity, innate immune cells, NK cells, tuberculosis

Introduction

Tuberculosis (TB), mainly caused by *Mycobacterium tuberculosis (Mtb)*, is the leading cause of death among infectious diseases. In 2018, an estimated 10 million people developed clinical TB and an estimated 2·2 billion people are *Mtb* infected globally [1]. Current control strategy in endemic countries depends upon passive case-finding and treatment of active cases, based on directly observed treatment short-course recommended by the World Health Organization (WHO) [2]. The United Nations sustainable development goal (target 3.3) aims at ending the TB epidemic by reducing TB-related deaths by 90% by 2030 [1]. However, such an ambitious goal may not be achieved without an efficacious vaccine. Bacille Calmette–Guérin (BCG) vaccine, developed a century ago, is effective against childhood (disseminated and miliary) TB. However, its protective efficacy against pulmonary TB varies from 0 to 80% in different populations, and efforts to develop a new vaccine to replace BCG have achieved little success, mainly because of incomplete knowledge on correlates of protective immunity. Moreover, more than 90% of *Mtb*-infected individuals do not develop clinical TB, implying that they are immune protected [3]. Several studies have shown that some household contacts do not acquire infection, despite

extensive exposure to *Mtb* [4–6]. These individuals clear infection early and are referred to as 'resisters'. The resister phenotype is defined based on negative test results of interferon-gamma (IFN-γ)-release assay (IGRA) and tuberculin skin test (TST). As the two tests depend on recall response (immunological memory), this early clearance is attributed to innate and innate-like immune cells [4,5]. These innate cells include monocytes/macrophages; dendritic cells (DCs), neutrophils and natural killer (NK) cells. There are also reports of innate-like T cells such as mucosal-associated invariant T cells, gamma-delta T cells [7] and invariant NK T cells [8] playing a role in early *Mtb* clearance. In recent years, NK cells have received increasing attention in controlling *Mtb* infection. NK cells express various surface markers that recognize *Mtb* cell components and *Mtb*-infected cells. These cells employ direct and indirect mechanisms to kill *Mtb* and infected cells. In this review, recent data on the role of NK cells on early *Mtb* clearance, with emphasis on longitudinal studies, will be presented.

Evidence of early *Mtb* clearance

Early clearance of *Mtb* infection is defined as the eradication of infective *Mtb* before the development of acquired immunity [5]. In the TB endemic setting, exposure to *Mtb* has at least three possible outcomes. There are individuals (5–10%) who develop clinical disease after primary infection. Another group of individuals (90%) acquire *Mtb* infection but do not develop clinical TB. These individuals are believed to have latent *Mtb* infection. LTB is defined clinically by a reactive TST, indicating a delayed-type hypersensitivity (DTH) response to intradermal injection of *Mtb*-derived purified proteins or a T cell response to *Mtb*-specific antigens in the absence of clinical and radiological findings [9]. Both innate and adaptive immune cells and their products are involved in controlling clinical TB in LTB infection [10–12]. However, some household contacts who are extensively exposed to *Mtb* remain negative to TST or IGRA. These individuals are resisters, and clear *Mtb* infection early before the development of acquired immunity [4,5].

Observation of persistent TST negativity of some individuals, despite heavy exposure to *Mtb*, was reported in nursing students as early as the 1940s [13]. Case–control studies in the 1960s showed the existence of resistant individuals to *Mtb* infection following extended exposure. This evidence comes from US personnel aboard a USS destroyer who shared the same confined environment with index cases for 6 months. In this case, seven of 308 (10%) developed active TB, while seven (10%) of the crew members remained negative for TST after 6 months in the same ship with index cases. Another example is an evaluation of nursing students in the pre-antibiotic era, which showed TST-negative individuals despite extended exposure to *Mtb* [14]. Differences in susceptibility to *Mtb* among close contacts of TB index cases was suggested from a systematic review report [6], where close to 50% of close contacts remained uninfected. Results of some early studies suggest that rates of resistance can be as high as 70% of heavily *Mtb*-exposed close contacts [15–17]. Although the proportion of resistant individuals reported above could be due to the inherent shortcomings of TST, the presence of *Mtb*-resistant household contacts in an endemic setting is unquestionable.

In recent years, several studies in different endemic communities (reviewed in [18]) have established persistently TST- or IGRA-negative individuals in longitudinal studies during a period of 2 years. In these studies, the proportion of individuals who cleared infection early (resisters) ranged from 3·4% in South Africa [19] to 26·8% in Uganda [20]. However, the true proportion of resisters in a given endemic community is yet to be established, as most of these studies used either TST or IGRA. These two tests depend upon recall response/immunological memory, and do not discriminate between clinical TB and *Mtb* infection or exposure to *Mtb* and non-environmental mycobacteria. Although commercially available IGRAs are more specific than TST, these two tests have inherent shortcomings in terms of sensitivity and specificity and test results are influenced by many factors, such as duration and intensity (closeness) of contact and quality of aerosol [4,21].

However, there are recent studies [22,23] that used both TST and IGRA to determine the true proportion of resisters longitudinally. For instance, a study in India [22] examined 799 household contacts in culture-confirmed TB index cases in 355 households at baseline, 4–6 months and 12 months. They found 52 (6·5%) in 39 households to be resisters. The authors found no epidemiological factors associated with the resister phenotype based on random-effects Poisson regression.

Another study, in Uganda [23], followed-up 407 HIV-negative household contacts for more than 2 years using both TST and IGRA. The study concluded that resistance to latent infection in adults, who have had close contact with pulmonary TB patients living in TB-endemic areas, is a stable outcome of *Mtb* exposure. Repeated longitudinal measurements with two different immune assays and extended follow-up provide enhanced discriminatory power to identify this resister phenotype and avoid misclassification [23].

Immunological basis of early *Mtb* clearance

Our knowledge of immune protection against *Mtb* infection is incomplete, especially concerning the resister phenotype. As early *Mtb* clearance occurs before the

development of acquired immunity, innate immune cells such as monocytes/macrophages, DCs, neutrophils and NK cells are believed to be responsible for this early response [4,5,24,25]. In the lungs, alveolar macrophages (AMs) are the first innate cells to encounter *Mtb*. However, the number of other mononuclear cell subsets recruited to the infected lungs increases by 20–30-fold following *Mtb* infection [26,27]. Examination of the distribution of *Mtb* in these cell subsets on day 14 post-infection shows that the pathogen was equally distributed in AMs, myeloid DCs and neutrophils [26]. Moreover, recent findings indicate that monocytes, DCs, neutrophils, epithelial cells, endothelial cells and fibroblasts are recruited to the lungs following cytokine signaling by AMs (reviewed in [11,12,28]).

While these innate cells are critical for early anti-mycobacterial responses, they are also targets of infection by the pathogen and serve as niches for bacterial replication, disease progression and dissemination of the pathogen to different organs and tissues [12,28]. Moreover, recruitment of neutrophils serves as an early line of defense against *Mtb* infection via secretion of anti-microbial molecules and inflammatory mediators. After recruitment, neutrophils recognize bacteria either directly through crystallizable fraction receptor (Fc-R), and complement receptor and phagocytosis the bacteria [29,30]. However, neutrophils also serve as niches for bacterial replication, can impede immunity against *Mtb* [12]) and mediate immunopathology during *Mtb* infection [12,30].

An innate immune cell that has attracted increasing attention in recent years in host defense against *Mtb* is the NK cell. NK cells belong to the same group of innate immune cells, such as monocytes/macrophages, DCs and neutrophils. Unlike the above innate phagocytic cells, NK cells do not serve as a niche for *Mtb* and do not disseminate the pathogen. This feature, combined with the strategic distribution in lymphoid non-lymphoid tissues and organs, make NK cells critically important in the fight against *Mtb*.

The biology of NK cells

NK cells constitute large granular lymphocytes and belong to group 1 of innate lymphoid cells (ILC1) [31] and express CD56 (neural cell adhesion molecule, NCAM) and lack CD3 and CD19 [32]. NK cells are distributed in the blood, lymphoid organs (including the thymus and spleen) and non-lymphoid organs (including the lungs, liver and the uterus, as well as tissues such as skin) [33–35]. In humans, NK cells subsets exhibit major functional differences in their cytotoxicity, cytokine production and homing capabilities [36]. Based on CD56 density on the cell surface, NK cells are comprised of types, namely, $CD56^{bright}$ and $CD56^{dim}$, which have different phenotypical properties. $CD56^{bright}$ NK cells have the capacity to produce large quantities of cytokines, while $CD56^{dim}$ NK cells are more cytotoxic and express more killer immunoglobulin (Ig)-like receptors (KIR) as well as Fc-ϒ receptor III (Fc-GR III, CD16) [36–38]. NK cells express activating and inhibitory receptors on their surface, which define their functional properties [34,35,39,40]. NK cells lyse target cells that express insufficient or lack MHC-1 molecules, whereas cells that express MHC-I molecule are not affected.

Inhibitory receptors

Inhibitory receptors specific for major histocompatibility complex (MHC)-1 antigens tightly regulate NK cell-mediated cytotoxicity and cytokine production. The inhibitory signal from the MHC-1-specific receptor is essential for hematopoietic target cells to avoid destruction by NK cells. This concept is termed 'missing self' [41–43]. Such MHC-1 recognizing inhibitory receptors form three families of NK cell surface receptors; namely, KIRs, LIRs (leukocyte Ig-like receptors) and NKG2A (natural killer group 2A) [34,35]. KIRs, which are members of the Ig superfamily, are type 1 transmembrane molecules that recognize classical human leukocyte antigens A, B and C (HLA class IA [44–46]. LIRs, also known as ILTs (Ig-like transcripts), form the second set of receptors and mainly recognize non-classical HLA-G (class IB) HLA class IA molecules. LIRs belong to the same Ig superfamily as KIRs. NKG2A, a member of the NKG2 group of seven receptors, namely A,B, C, D, E, F and H, dimerize with CD94 to form the NKG2A/CD94 receptor. It belongs to the c-type lectin family of receptors that recognizes non-classical HLA-E class I molecule as its ligand [47].

Activating receptors

Natural cytotoxicity receptors (NCRs) represent the group of NK cell surface-activating receptors that include NKp46, NKp30 and NKp44. Nkp46, also known as natural cytotoxicity receptor 1 (NCR1), is a 46-kDa transmembrane protein belonging to the Ig superfamily. In humans, NKp46 is expressed by all $CD56^{dim}$ $CD16^{+}$ and $CD56^{bright}$ $CD16^{-}$ human NK cells, irrespective of their activation status [48]. NKp30, also called natural cytotoxicity receptor 3 (NCR3), is a 30-kDa protein expressed on all mature resting and activated NK cells [49]. These receptors, as well as NKG2D, recognize ligands expressed on target cells [50–52]. CD16 (Fc-ϒR III), also an activating receptor, is expressed mainly by the $CD56^{dim}$ NK cell subset and is essential for ADCC against IgG-coated target cells [37,53].

Mtb recognition by NK cells

NK cells use non-antigenic specific mechanisms to exert effector functions, and pattern recognition receptors recognize pathogen-associated molecular patterns and are essential components of the NK cell-mediated innate immune response against *Mtb* [34,40]. Various components of *Mtb* cell wall can bind directly to NKp44 of NK cells [54], and NK cells can recognize stress molecules upregulated on the surface of *Mtb*-infected cells [55]. For example, Nkp44 directly binds to *Mtb* cell wall components, such as arabinogalactan–peptidoglycan, as well as mycolic acids and arabinogalactan derivatives [54,56]. Moreover, NKp46 was reported to play a dominant role in the lysis of mononuclear phagocytes infected with *Mtb* via recognition of vimentin expressed on the surface of *Mtb*-infected cells [55,57].

Human NK cells directly recognize *Mtb* by the binding of TLR-2 and NKp44 to peptidoglycan and other components of the cell wall, respectively, and become activated [54,56,58]. In one study, in T cell-deficient mice, it was demonstrated that NK cells mediated early defense against *Mtb* infection via interferon (IFN)-γ [59,60]. In humans, NK cells in the peripheral blood stimulated with *Mtb* or BCG upregulated IFN-γ expression [61,62].

Evidence of NK cell response to *Mtb* from epidemiological studies

One earlier study has shown that the pleural fluid of TB patients was enriched with IFN-γ-producing $CD56^{bright}$ NK cells due to selective apoptosis of cytotoxic $CD56^{dim}$ NK cells induced by soluble factors present in TB effusions [63]. A longitudinal study on a cohort of South African adolescents found that the frequency of NK cells in the peripheral blood could inform disease progression, therapeutic response and lung inflammation of patients with active TB. This group has shown that NK cells from individuals with LTB display elevated levels of cytotoxicity and increased frequency of NK cells [64]. In a study carried out to assess the contribution of NK cells against *Mtb* infection, a cross-sectional assessment of NK cell phenotype and function in four distinct groups (pretreatment TB patients, post-treatment TB patients, household contacts and TST-negative) of individuals was made. The results showed a significant decrease in IFN-γ expression and degranulation in NK cells of TB patients, with no variation in NK cell frequencies. Moreover, CD57 expression, a marker for advanced NK cell differentiation, was significantly lower in cases post-treatment compared to pretreatment. NKG2C, an activation marker and imprinted-memory marker, has significantly increased in TST^+ (latently infected) compared to TB cases and TST-resistant individuals [64].

In a recent study in China, using single-cell RNA sequencing of peripheral blood mononuclear cells (PBMC) from household contacts (HHC), LTB-infected and TB patients, it was found that infection with *Mtb* changed the frequency of immune cell subsets. In particular, there was a gradual depletion of the NK cell subset [$CD3^-CD7^+$ granzyme B ($GzmB^+$)] from HHC to LTB and TB. NK frequency in TB patients was restored following anti-TB treatment [65].

Moreover, to determine NK cell phenotype and functional responses to *Mtb* using flow cytometry, Harris *et al.* [66] compared three groups: Quantiferon (QFT)-positive and -negative adults in TB endemic setting in Kisumu, Kenya, and compared NK cell responses to those of *Mtb*-naive healthy adult controls in the United States. The results showed a distinct $CD56^{dim}$ NK cell phenotype that differentiated the Kenyan and US groups. In addition, among Kenyan participants, NK cells from QFT-positive individuals with latent *Mtb* infection were characterized by significant down-regulation of NKp44 and the inhibitory receptor T cell immunoreceptor with Ig and ITIM domains (TIGIT) compared with QFT-negative individuals. Moreover, the distinct $CD56^{dim}$ phenotypical profiles in Kenyan individuals correlated with dampened NK cell responses to tumor cells and diminished activation, degranulation and cytokine production following stimulation with *Mtb* antigens, compared with *Mtb*-naive US healthy adult controls. Together, these data provide evidence that phenotypical and functional profiles of NK cells are modified in TB-endemic settings [66].

Possible mechanisms of *Mtb* killing by NK cells

NK cells use two suggested mechanisms in controlling *Mtb* infection: direct and indirect. First, NK cells are cytotoxic lymphocytes that lyse cells infected with intracellular pathogens [67]. The cytolytic function of NK cells can be initiated primarily through degranulation and death receptor ligation, and is critical for the clearance of diseased and dysfunctional cells [68,69]. Secondly, NK cells can produce a variety of inflammatory cytokines in response to activation receptor stimulation, as well as inflammatory cytokine-induced activation signaling [70,71].

Direct mechanisms

Direct mechanisms of NK cell-mediated control of infection follow three steps: (1) target cell recognition, (2) target cell contact and immunological synapse (IS) formation and (3) NK cell-induced target cell death [34]. The main direct mechanism of NK cell cytotoxicity is through cytoplasmic granules containing perforin, granulysin and granzymes, as well as several death receptors that can initiate apoptosis (Fig. 1). Perforin belongs to the membrane attack

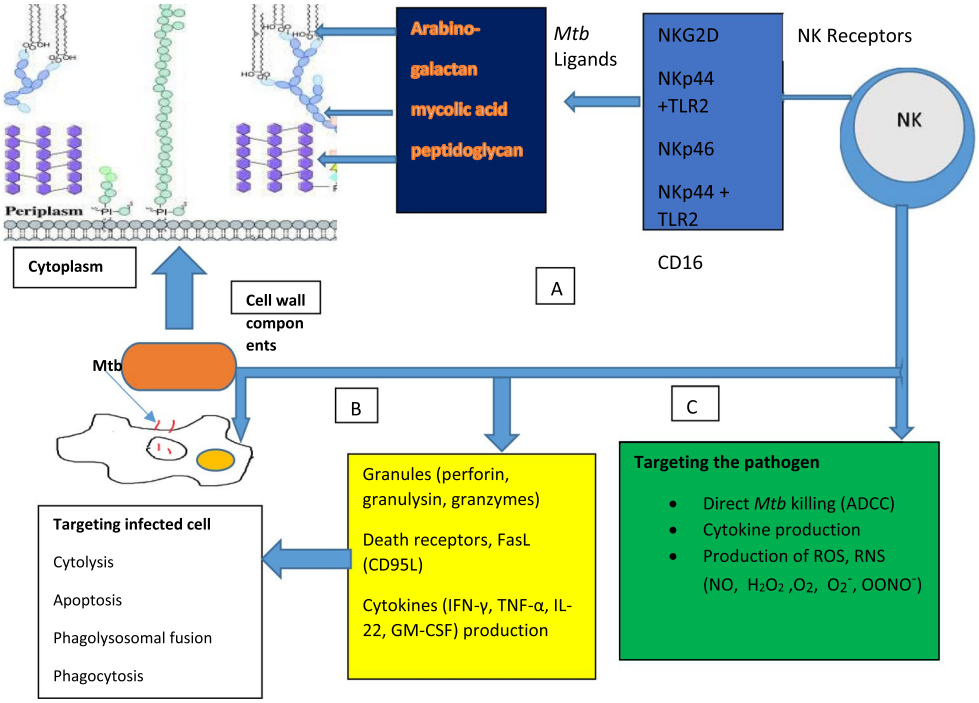

Fig. 1. An overview of natural killer (NK) receptors, *Mycobacterium tuberculosis* (*Mtb*) ligands and mechanisms employed by NK cells to kill either *Mtb* or *Mtb*-infected cells. (a) NK cells have receptors [NKp44, NKp46, Toll-like receptor (TLR-2), NK group 2D (NKG2D)] that bind directly to cell wall components (e.g. arabinogalactan, mycolic acid, peptidoglycan) of *Mtb*. (b) Targeting infected cells: through the release of cytoplasmic granules (perforin, granulysin, and granzyme), NK cells cause lysis of infected cells; through ligation of FasL and Fas on infected cells NK cells induce apoptosis, and through cytokine production [interferon (IFN)-γ, tumor necrosis factor (TNF)-α, interleukin (IL-22)], NK cells promote maturation of phagolysosome and promote phagocytosis. (c) Directly targeting the pathogen: by binding cell components via TLR-2 and cytotoxic receptors (e.g. NKp44), NK cells can kill the pathogen through antibody-dependent cellular cytotoxicity and the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) ($H2O2, O2^{-}, OONO^{-}, NO, O^{-}$).

complex protein family, and inserts into target cell membrane to function as a pore similar to the C5–9 membrane attack complex of the complement system [72]. Perforin pores are used to facilitate transport of granulysin and granzyme into the target cell cytoplasm. Granzymes are a family of serine proteases with many members, the major constituent in NK cells being granzyme B. This enzyme can initiate apoptosis of the target cell through direct activation of caspases 3 and 7 or proteolysis of the protein BH3-interacting domain death agonist (Bid). Cleavage of Bid allows the active fragment to move to the mitochondrial membrane and form a pore complex with Bax and Bak, promoting the exit of cytochrome c into the cytosol, thereby initiating the formation of a caspase-activating complex [73]. Granzyme B can also mediate nuclear destruction in the presence of perforin, and granzymes may be able to mediate caspase-independent cell death pathways [69].

Another direct mechanism of cell NK cell cytolysis is through apoptosis. Fas (CD95) is a tumor necrosis factor (TNF) receptor family transmembrane death receptor responsible for cell lysis, whose ligand (FasL) is expressed in NK cells [74,75]. The Fas receptor can be found on most cell types in the body, and is of particular interest in the context of macrophage expression. Upon Fas–FasL ligation a death-inducing signaling complex (DISC) forms, composed of multiple proteins, including Fas, Fas-associated death domain (FADD) and caspase-8. Activation of caspase-8 by DISC initiates the extra mitochondrial apoptotic pathway [75]. Macrophages infected with *Mtb* undergo NK-mediated apoptosis through this Fas pathway to limit viability of *Mtb* [76].

Thirdly, NK cells express CD40L, the CD40 ligand that is expressed on antigen-presenting cells (APCs) [77,78]. After ligation, CD40 leads to an up-regulation of co-stimulatory molecules CD80 and CD86 on the cell surface of APCs as well as generation of nitric oxide (NO) when accompanied by IFN-γ [79].

Moreover, there are reports that glutathione (GSH), which is a non-protein thiol, controls intracellular *Mtb* growth through different mechanisms. It has been shown that low levels of intracellular GSH decrease NK cytotoxic function. Moreover, increased GSH inhibits intracellular *Mtb* (H37Rv) within human monocyte-derived

macrophages through redox imbalance and by a structural similarity to penicillin (reviewed in [80]).

NK cell-mediated proinflammatory cytokine production

Upon stimulation by *Mtb* antigens, NK cells produce pro-inflammatory cytokines (Fig. 1), such as IFN-γ, TNF-α, IL-22 and granulocyte–monocyte colony-stimulating factor (GM-CSF), and these cytokines exert their effects on infected cells. For instance, IFN-γ released by NK cells can trigger numerous intracellular effector mechanisms within macrophages, such as activation of nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase type 1 and 2 (NOX1.2) as well as NO synthase type 2, leading to the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), respectively [81,82]. Superoxide can spontaneously generate hydrogen peroxide (H2O2) and hydroxyl (HO^-) radicals [83,84]. ROS and RNS can react further with each other to generate NO2 and peroxynitrate ($OONO^-$) [83,85,86]. These various reactive species contribute to anti-microbial oxidative destruction to membrane lipids and proteins, DNA and enzymes [83]. IFN-γ also up-regulates the expression of IgG Fc-γ-RI in monocytes to increase opsonization-dependent phagocytosis [81]. NOX2 is recruited to phagosomes by IFN-γ stimulation, where it catalyzes the production of superoxide ($O2^-$) from O2 and NADPH [84,87]. NOS2 catalyzes the conversion of L-arginine and O2 into NO and citrulline [87].

Secondly, TNF-α released by NK cells contributes to macrophage mitochondrial ROS formation through a TNF-α receptor (TNFR1) complex association with NOX1, induction of NOX2 and a receptor-interacting serine-threonine kinase 1- and 3-dependent pathway, which can lead to programmed necrosis [88–90].

Thirdly, some studies have shown that human NK cells produce IL-22 in addition to IFN-γ and TNF-α. IL-22 is a member of the IL-10 cytokine family that is produced by special immune cell populations, including $CD4^+$ and $CD8^+$ T cells, which display either a protective or a pathogenic role in chronic inflammatory diseases [91]. IL-22 plays an important role in host defense and homeostasis through production of anti-microbial peptides [92], and has been shown *in vitro* to inhibit *Mtb* intracellular growth by enhancing phagolysosomal fusion [93].

Antibody-dependent NK cell cytotoxicity

It is generally believed that early *Mtb* clearance is conferred by innate cells before the development of adaptive immunity. However, antibody-responsive innate immune cells bearing Fc-R have been reported in TB granulomas, suggesting that they may play a role in the anti-microbial response. For instance, it has been shown that antibodies against *Mtb* lipoarabinomannan enhance bacterial opsonization and restrict intracellular growth [94]. Beyond opsonization, antibodies direct innate immune anti-microbial activity via their constant Fc domains following engagement of Fc-R found on all innate immune cells [94]. Antibodies enhance cytolysis of infected target cells by NK cells and complement. Using an unbiased antibody profiling approach. Lu *et al*. [94] have shown that individuals with LTB and active TB have distinct *Mtb*-specific antibody responses, such that LTB infection is associated with unique antibody Fc functional profiles, selective binding to Fc-γ-RIII and distinct antibody glycosylation patterns. A recent cohort study in Uganda by Lu *et al*. [95] reported that resisters possess IgM, class-switching IgG antibody responses and non-IFN-γ T cell responses to *Mtb*-specific proteins early-secreted antigen target-6 and culture filtrate protein 10. Compared to subjects with classic LTB, resisters displayed enhanced antibody avidity and distinct *Mtb*-specific IgG Fc profiles.

Conclusions

In endemic communities there are household contacts of index cases who, despite extensive exposure to *Mtb*, persistently remain TST- and/or IGRA-negative. These individuals, referred to as 'resisters', are believed to clear *Mtb* infection early before the development of adaptive immunity. Although several innate immune cells and innate-like T cells are involved in response to *Mtb* infection, NK cells are increasingly recognized as playing a vital role in defense against *Mtb* infection. NK cells are strategically distributed in lymphoid and non-lymphoid tissues and organs, including the lungs, which makes them increasingly relevant to early *Mtb* protection. NK cells non-specifically bind to *Mtb* cell wall components through various receptors (TLR-2, NKp46, NKp30, NKp44, NKG2D and CD16). NK cells can also recognize *Mtb*-infected cells through up-regulated IFN-γ expression and KIR and NKG2D receptors. Moreover, NK cells kill the pathogen and infected cells using different mechanisms, including destroying infected cells via cytolysis, apoptosis, GSH and production of cytokines (IFN-γ, TNF-α and IL-22). Moreover, there are also NK cell mechanisms that target the pathogen, including antibody-dependent NK cell cytotoxicity and generation of reactive nitrogen and oxygen species. There is convincing evidence from cohort studies in endemic communities that NK cells are involved in early *Mtb* clearance. Thus, the above attributes of NK cells will make them ideal for future research in vaccine design and development against *Mtb* infection.

Acknowledgements

None.

Disclosures

The authors have no conflicts of interest to declare.

Data Availability Statement

Not applicable, as this is a review paper.

References

1 World Health Organization (WHO). Global tuberculosis report, 2019. Geneva, Switzerland: WHO, 2019: 1–297. Available at: https://www.who.int/teams/global-tuberculosis-programme/tb-reports
2 World Health Organization (WHO). Global tuberculosis report, 1997. Geneva, Switzerland: WHO, 1997: 1–16. Available at: https://apps.who.int/iris/bitstream/handle/10665/63548/WHO_TB_97.228.pdf;jsessionid=89F5F333D76582EEDF91E8C68EC0C73B?sequence=1
3 Doherty M, Wallis RS, Zumla A. Biomarkers for tuberculosis disease status and diagnosis. Curr Opin Pulm Med 2009; **15**:181–7.
4 Simmons JD, Stein CM, Seshardi C *et al*. Immunological mechanisms of human resistance to persistent *Mycobacterium tuberculosis* infection. Nat Rev Immunol 2018; **18**:575–89.
5 Verrall AJ, Netea MG, Alishahbana B *et al*. Early clearance of *Mycobacterium tuberculosis*: a new frontier in prevention. Immunology 2013; **141**:506–13.
6 Morrison J, Pai M, Hopewell PC. Tuberculosis and latent tuberculosis infection of close contacts of people with pulmonary tuberculosis in low income and middle-income countries: a systematic review and meta-analysis. Lancet Infect Dis 2008; **8**:359–66.
7 Kyriakos-Vorkas C, Wipperman MF, Li K *et al*. Mucosal-associated invariant and gd T cell substes respond to initial *Mycobacterium tuberculosis* infection. JCI Insight 2018; **3**:e121899.
8 Trottein F, Paget C. Natural killer T cells and mucosal-associated invariant T cells in the lung infections. Front Immunol 2018; **9**:1750.
9 Horsburgh CR Jr, Rubin EJ. Latent tuberculosis infection in the United States. N Eng J Med 2011; **364**:1441–8.
10 Dutta NK, Karakousis C. Latent tuberculosis infection: myths, models, and mechanisms. Microbiol Mol Biol Rev 2014; **78**:343–71.
11 Bussi G, Gutierrez MG. *Mycobacterium tuberculosis* infection of host cells in space and time. FEMS Microbiol Rev 2019; **43**:341–61.
12 Sia JK, Rengarajan J. Immunology of *Mycobacterium tuberculosis* infections. Microbiol Spectr 2019; **7**. https://doi.org/10.1128/microbiolspec.GPP3-0022-2018.
13 Israel HL, Hetchington HW, Ord IG. A study of tuberculosis among students of nursing. JAMA 1941; **194**:839–44.
14 Houk VN, Baker JH, Sorensen K, Kent DC. The epidemiology of tuberculosis in a closed environment. Arch Environ Heath 1968; **16**:26–35.
15 Hill PC, Brookes RH, Fox A *et al*. Longitudinal assessment of an ELISPOT test for *Mycobacterium tuberculosis* infection. PLOS Med 2007;**4**:e192.
16 Lemos AC, Matos ED, Pedral-Sampaio DB *et al*. Risk of tuberculosis among household contacts in Salvador, Bahia. Braz J Infect Dis 2004; **8**:424–30.
17 Devadatta S, Dawson JJY, Fox W *et al*. Attack rate of tuberculosis in a 5-year period among close family contacts of tuberculous patients under domiciliary treatment with isoniazid plus PAS or isoniazid alone. Bull World Health Org 1970; **42**:337–51.
18 Meermeir EW, Lewinsohn DM. Early clearance versus control: what is the meaning of a negative tuberculin skin test or interferon-gamma release assay following exposure to *Mycobacterium tuberculosis*? F1000Research 2018; **7**:664.
19 Cobat A, Gallant CJ, Simkin L *et al*. Two loci control tuberculin skin test reactivity in an area hyperendemic for tuberculosis. J Exp Med 2009; **206**:2583–91.
20 Bark CM, Manceur A, Malone LL *et al*. Identification of host proteins predictive of early stage *Mycobacterium tuberculosis* infection. EBioMedicine 2017; **21**:150–7.
21 Yones-Lopez EC, Acuna-Villaorduna C, Fregona G *et al*. Incident *Mycobacterium tuberculosis* infection in household contacts of infectious tuberculosis patients in Brazil. BMC Infect Dis 2017; **17**:576. https://doi.org/10.1186/s12879-017-2675-3.
22 Mave V, Chandrasekaran P, Chavan A *et al*. Infection free 'resisters' among household contacts of adult pulmonary tuberculosis. PLOS ONE 2019; **14**:e0218034.
23 Stein CM, Zalawango S, Malone LL *et al*. Resistance and susceptibility to Mycobacterium tuberculosis infection and disease in tuberculosis households in Kampala, Uganda. Am J Epidemiol 2019; **187**:1477–89.
24 Lin PP, Flynn J. The end of the binary era: revising the spectrum of tuberculosis. J Immunol 2018; **201**:2541–8.
25 Keiser TL, Purdy GE. Killing *Mycobacterium tuberculosis in vitro*: what model systems can teach us? Microbiol Spectr 2017; **5**. https://doi.org/10.1128/microbiolspec.TBTB2-0028-2016.
26 Wolf AJ, Linas B, Trevejo-Nuñez GJ *et al*. *Mycobacterium tuberculosis* infects dendritic cells with high frequency and impairs their function *in vivo*. J Immunol 2007; **179**:2509–19.
27 Skold M, Behar SM. Tuberculosis triggers a tissue-dependent program of differentiation and acquisition of effector functions by circulating monocytes. J Immunol 2008; **181**:6349–60.
28 Lerner TR, Borel S, Greenwood DJ *et al*. *Mycobacterium tuberculosis* replicates within necrotic human macrophages. J Cell Biol 2017; **216**:583–94.
29 Witko-Sarsat V, Rieu P, Descamps-Latscha B, Lesavre P, Halbwachs-Mecarelli L. Neutrophils: molecules, functions, and pathophysiological aspects. Lab Invest 2000; **80**:617–653.

30 Lyadova IV. Neutrophils in tuberculosis; heterogeneity shapes the way? Mediat Inflamm 2017; **2017**:1–11.

31 Spits H, Artis D, Colonna M *et al*. Innate lymphoid cells – a proposal for uniform nomenclature. Nat Rev Immunol 2013; **13**:145–9.

32 Trinchieri G. The biology of natural killer cells. Adv Immunol 1989; **47**:187–376.

33 Gong J, Wei H. Natural killer cells in the lungs. Front Immunol 2019; **10**:1416–29.

34 Abel AM, Yang C, Thakar MS, Malarkannan S. Natural killer cells: development, maturation, and clinical utilization. Front Immunol 2018; **9**:1869.

35 Khan M, Arroj S, Wang H. NK cell-based immune checkpoint inhibition. Front Immunol 2020; **11**:167.

36 Moretta A, Marcenaro E, Parolini S *et al*. NK cells at the interface between innate and adaptive immunity. Cell Death Diff 2008; **15**:226–33.

37 Cooper MA, Fehinger TA, Caliguiri MA *et al*. The biology of human natural killer-cell subsets. Trends Immunol 2001; **22**:633–40.

38 Fu B, Tian Z, Wei H. Subsets of human natural killer cells and their regulatory effects. Immunology 2014; **141**:483–9.

39 Hashemi E, Malarkannan S. Tissue resident NK cells: development, maturation, and clinical relevance. Cancers 2020; **12**:1553.

40 Barrow AD, Martin CJ, Colonna M *et al*. Natural cytotoxicity receptors in health and disease. Front Immunol 2019; **10**:909. https://doi.org/10.3389/fimmu.2019.00909.

41 Moretta I, Bottino C, Pende D, Vitale M, Mingari MC, Moretta A. Different checkpoints in human NK cell activation. Trends Immunol 2004; **25**:670–6.

42 Long EO. Regulation of immune responses through inhibitory receptors. Annu Rev Immunol 1999; **17**:875–904.

43 Long EO. Negative signaling by inhibitory receptors: the NK cell paradigm. Immunol Rev 2008; **224**:70–84.

44 Waktmann N, Rajagopalan S, Winter CC, Peruzzi M, Long EO. Killer cell inhibitory receptors specific for HLA-C and HLA-B identified by direct binding and by functional transfer. Immunity 1995; **3**:801–9.

45 Moretta A, Mingari MC, Pende D *et al*. The molecular basis of natural killer (NK) cell recognition and function. J Clin Immunol 1996; **16**:243–53.

46 Moretta A, Biassoni R, Bottino C *et al*. Major histocompatibility complex class-1 specific receptors on human natural killer and T lymphocytes. Immunol Rev 1997; **155**:105–17.

47 Borrego F, Kabat K, Jim DK *et al*. Structure and function of major histocompatibility complex (MHC) class-1 specific receptors expressed on human natural killer (NK) cells. Mol Immunol 2002; **38**:637–60.

48 Sivori S, Vitale M, Morelli I *et al*. P46, a novel natural killer cell-specific surface molecule that mediates cell activation. J Exp Med 1997; **186**:1129–36.

49 Pende D, Parolini S, Pessino A *et al*. Identification and molecular characterization of NKp30, a novel triggering receptor involved in natural cytotoxicity mediated by human natural killer cells. J Exp Med 1999; **190**:1505–16.

50 Moretta A, Botino C, Vitale M *et al*. Activating receptors and co-receptors involved in human natural killer cell-mediated cytolysis. Annu Rev Immunol 2001; **19**:192–223.

51 Moretta I, Montaldo E, Vacca P *et al*. Human natural killer cells: origin, receptors, function and clinical applications. Int Arch Allergy Immunol 2014; **164**:253–64.

52 Vitale M, Cantoni C, Della Cheisa M *et al*. An historical overview: the discovery of how NK cells can kill enemies, recruit defense troops, and more. Front Immunol 2019; **10**:1415.

53 Wang W, Erbe AK, Hank JA, Morris ZS, Sondel PM. NK cell-mediated antibody-dependent cellular cytotoxicity in cancer immunotherapy. Front Immunol 2015; **6**:368.

54 Esin S, Counoupas C, Aulocino A *et al*. Interaction of mycobacterium cell wall components with the human natural killer receptor NKp44 and toll-like receptor 2. Scand J Immunol 2013; **77**:460–9.

55 Vankayalapati R, Garg A, Porgador A *et al*. Role of NK cell activating receptors and their ligands in the lysis of mononuclear phagocytes infected with an intracellular bacterium. J Immunol 2005; **175**:4611–7.

56 Esin S, Batoni G, Counoupas C *et al*. Direct binding of human NK cell natural cytotoxicity receptor NKp44 to the surfaces of mycobacteria and other bacteria. Infect Immun 2008; **76**:1719–27.

57 Garg A, Barnes PF, Porgador A *et al*. Vimentin expressed on *Mycobacterium tuberculosis*-infected human monocytes is involved in binding to the NKp46 receptor. J Immunol 2006; **175**:6192–8.

58 Marcnero E, Ferranti B, Falco M, Moretta A. Human NK cell directly recognizes *Mycobacterium bovis* via TLR2 and acquire the ability to kill monocyte-derived DC. Int Immunol 2008; **20**:1155–67.

59 Feng CC, Kaviratine M, Rothfuchs AG *et al*. NK cell-derived IFN-γ differentially regulates innate resistance and neutrphil response in T cell deficient hosts infected with *Mycobacterium tuberculosis*. J Immunol 2006; **177**:7086–93.

60 Liu CH, Liu H, Ge B. Innate immunity in tuberculosis: host defense and pathogen evasion. Cell Mol Immunol 2017; **14**:963–75.

61 Gerosa F, Baldani-Guerra B, Nissi C, Marchesini V, Carra G, Trinchieri G. Reciprocal activation interaction between natural killer cells and dendritic cells. J Exp Med 2002; **195**: 327–33.

62 Feinberg J, Fieschi C, Doffinger R *et al*. Bacille Calmette–Guérin triggers the IL-12/IFN-gamma axis by IRAK-4 and NEMO-dependent non-cognate interaction between monocytes, NK and T lymphocytes. Eur J Immunol 2004; **34**:3276–84.

63 Schierloh P, Yokobori N, Aleman M *et al*. Increased susceptibility to apoptosis of CD56 dim Cd16+ NK cells induces the enrichment of IFN-producing of CD56 bright cells in tuberculosis peurisy. J Immunol 2005; **175**:6852–60.

64 Chowdhury RR, Vallania F, Yang Q *et al*. A multi-cohort study of the immune factors associated with *M. tuberculosis* infection outcomes. Nature 2018; **560**:644–8.

65 Cai Y, Dai Y, Wang Y *et al*. Single-cell transcripotmics of blood reveals anatural cell subset depletion in tuberculosis. EBiomedicine 2020; **53**:102686. https://doi.org/10.1016/j.eboim.2020.102686.

66 Garand M, Goodlier M, Owolabi O *et al*. Functional and phenotypic changes of natural killer cells in whole blood during *Mycobacterium tuberculosis* infection and disease. Front Immunol 2018; **9**:257. https://doi.org/10.3389/fimmu.2018.00257.

67 Zhang Y, Huang B. The development and diversity of ILCs, NK cells and their relevance in health and disease. Adv Exp Med Biol 2017; **1024**:225–44.

68 Stabile H, Fionda C, Gismondi A, Santoni A. Role of distinct natural killer cell subsets in anticancer response. Front Immunol 2017; **8**:293.

69 Symth SJ, Cretney E, Kelly JM *et al*. Activation of NK cell cytotoxicty. Mol Immunol 2005; **42**:501–10.

70 Fauriat C, Long EO, Ljungggren H-G, Bryceson Y. Regulation of human NK-cell cytokine and chemokine production by target cell recognition. Blood 2010; **115**:2167–76.

71 Freeman RE, Raue HP, Hill AB, Mk S. Cytokine mediated activation of NK cells during viral infection. J Virol 2015; **89**:7922–31.

72 Voskoboinik I, Dunestone MA, Baran K, Whisstock JC, Trapani JA. Perforin: structure, function, and role in human immunopathology. Immunol Rev 2010; **235**:35–54.

73 Afonina IS, Cullen SP, Martin SJ. Cytotoxic and non-cytotoxic roles of CTL/NK protease granzyme B. Immunol Rev 2010; **235**:105–16.

74 Oshimi Y, Odda S, Honda Y, Nagata S, Miyazaki S. Involvement of Fas ligand and Fas-mediated pathway in the cytotoxicity of human natural killer cells. J Immunol 1996; **157**:2909–15.

75 Bao Q, Shi Y. Apoptosome: a pltaform for for the activation of initiator caspases. Cell Death Differ 2007; **14**:56–65.

76 Oddo M, Renno T, Attinger A, Bakker T, Macdonald HR, Melan PR. Fas ligand induced apoptosis of infected human macrophages reduces the viability of intracellular *Mycobacterium tuberculosis*. J Immunol 1998; **160**:5448–54.

77 Alderson MR, Armitage RJ, Tough TW, Strockbine I, Fanslow WC, Spriggs MK. CD40 expression by human monocytes: regulation by cytokines and activation of monocytes by the ligand for CD40. J Exp Med 1993; **178**:669–74.

78 Carbone E, Ruggiero G, Terrazanno G *et al*. A new mechanism of NK cell cytotoxicity activation: the CD40–CD40 ligand interaction. J Exp Med 1997; **185**:2053–60.

79 Suttles J, Stout RD. Macrophage CD40 signaling: a pivotal regulator disease protection and pathogenesis. Semin Immunol 2009; **21**:257–64.

80 Allen M, Balley C, Cahatol I *et al*. Mechanisms of control of Mycobacterium tuberculosis by NK cells: role of glutathione. Front Immunol 2015; **6**:508. https://doi.org/10.3389/fimun.2015.00508.

81 Schroder K, Hertzog PJ, Ravasi T, Hume DA. Interferon-gamma: an overview of signals, mechanisms of function. J Leuc Biol 2004; **75**:163–9.

82 Kuwano Y, Kawahara T, Yamamoto H *et al*. Interferon-γ activates transcription of NADH oxidase 1 gene and upregulates production of superoxide anion by human large intestinal epithelial cells. Am J Physiol Cell Physiol 2006; **290**:C433–C443.

83 Weiss G, Schaible UE. Macrophage defense mechanisms against intracellular bacteria. Immunol Rev 2015; **264**:182–203.

84 Lambeth JD. NOX enzymes and the biology of reactive oxygen. Nat Rev Immunol 2004; **4**:181–9.

85 Nathan C, Shiloh MU. Reactive oxygen and nitrogen intermediates in the relationship between mammalian host and microbial pathogens. Proc Natl Acad Sci USA 2000; **97**:8841–48.

86 Venkettarmann V, Dayaram YK, Talaue MT, Connel ND. Glutathione and nitrosoglatuthione in macrophage defense against *Mycobacterium tuberculosis*. Infect Immun 2005; **73**:1886–9.

87 Bodan C. Nitric oxide and the immune response. Nat Immunol 2001; **2**:907–16.

88 Vandenabeele P, Galluzi I, Vanden Berghe T, Kroemer G. Molecular mechanisms of necroptosis: an ordered cellular explosion. Nat Rev Med Cell Biol 2010; **11**:700–14.

89 Roca FJ, Ramakrishnan L. TNF dually mediates resistance and susceptibility to mycobacteria via mitochondrial reactive oxygen species. Cell 2013; **153**:521–34.

90 Miller JI, Velmurugan K, Cowan MJ, Birken V. The type 1 NADH dehydrogenase of *Mycobacterium tuberculosis* counters phagosomal NOX2 activity to inhibit TNF-α mediated host cell apoptosis. PLOS Pathog 2010; **6**:e1000864. https://doi.org/10.1371/journal.ppat.1000864.

91 Fu X, Yu S, Yang B, Lao S, Li B, Wu C. Memory-like antigen-specific human NK cells from TB pleural fluids produced IL-22 in response to IL-15 or *Mycobacterium tuberculosis*. PLOS ONE 2016; **11**:e0151721. https://doi.org/10.1371/journal.pone.0151721.

92 Cella M, Fuchs A, Vermi W *et al*. A human natural killer cell subset provides an innate source of IL-22 for mucosal immunity. Nature 2009; **457**:722–5.

93 Dhiman R, Indramohan M, Barne PF *et al*. IL-22 produced by human NK cells inhibits growth of Mycobacterium tuberculosis by enhancing phagolysosomal fusion. J Immunol 2005; **183**:6639–45.

94 Lu LL, Chuang AW, Rosebrock T. A functional role of antibodies in tuberculosis. Cell 2016; **167**: 433–443e14.

95 Lu LL, Smith MT, Yu KKQ *et al*. IFN-γ-independent immune markers of *Mycobacterium tuberculosis* exposure. Nat Med 2019; **25**:977–87.