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IL-22 Produced by Human NK Cells Inhibits Growth of *Mycobacterium tuberculosis* by Enhancing Phagolysosomal Fusion

Rohan Dhiman,*† Mohanalaxmi Indramohan,*† Peter F. Barnes,*†‡ Ramesh C. Nayak,§ Padmaja Paidipally,*† L. Vijaya Mohan Rao,§ and Ramakrishna Vankayalapati2*†

We determined whether human NK cells could contribute to immune defenses against *Mycobacterium tuberculosis* through production of IL-22. CD3+CD56+ NK cells produced IL-22 when exposed to autologous monocytes and γ-irradiated *M. tuberculosis*, and this depended on the presence of IL-15 and IL-23, but not IL-12 or IL-18. IL-15-stimulated NK cells expressed 10.6 times more IL-22 than control NK cells, and IL-23 siRNA inhibited IL-15-mediated IL-22 production by NK cells. Soluble factors produced by IL-15-activated NK cells inhibited growth of *M. tuberculosis* in macrophages, and this effect was reversed by anti-IL-22. Addition of rIL-22 to infected macrophages enhanced phagolysosomal fusion and reduced growth of *M. tuberculosis*. We conclude that NK cells can contribute to immune defenses against *M. tuberculosis* through production of IL-22, which inhibits intracellular mycobacterial growth by enhancing phagolysosomal fusion. IL-15 and DAP-10 elicit IL-22 production by NK cells in response to *M. tuberculosis*. *The Journal of Immunology*, 2009, 183: 6639–6645.

**T**uberculosis is a leading cause of death from infectious diseases worldwide, claiming an estimated 1.9 million lives worldwide annually (1, 2). Multidrug-resistant tuberculosis continues to spread in many parts of the world, requiring prolonged therapy with potentially toxic agents that provide much lower cure rates, compared with that for drug-susceptible tuberculosis. Development of methods to enhance innate defenses against *Mycobacterium tuberculosis* is an attractive means to protect against both drug-resistant and drug-susceptible tuberculosis.

NK cells can kill autologous infected cells without prior sensitization, and are believed to play a central role in innate immunity to microbial pathogens. NK cells mediate protection against viruses, bacteria, and parasites (3–7), through destruction of infected cells and by secretion of cytokines that shape the adaptive immune response (3, 4, 7, 8). Human NK cells use the NKp46 and NKG2D receptors to lyse *M. tuberculosis*-infected monocytes and alveolar macrophages (9, 10). Human NK cells also produce IFN-γ when exposed to *M. bovis* BCG (11), and the pleural fluid of tuberculosis patients is enriched for CD56brightCD16− NK cells, which are the predominant source of IFN-γ (12). In mouse model, NK cells are activated and produce IFN-γ during the early response to pulmonary tuberculosis (13) and NK cell-derived IFN-γ regulates the anti-mycobacterial resistance mediated by neutrophils (14).

Recent studies have found that NK cells produce not only IFN-γ, but also IL-22 (15), a member of the IL-10 family of cytokines that is produced by T cells and NK cells (15–17) in response to IL-23 secreted by APCs (18–20). IL-22-producing cells at mucosal surfaces play an important role in host defense and homeostasis (15, 17, 21–24) and are essential for induction of antimicrobial peptides in response to bacterial infections (15, 25). In human *M. tuberculosis* infection, memory like CD4+ cells produce IL-22, and high IL-22 levels were present in bronchoalveolar lavage fluid of tuberculosis patients, compared with those in healthy donors (26).

In the current study, we determined whether NK cells could contribute to defenses against *M. tuberculosis* through production of IL-22. We found that human NK cells produce IL-22 in response to *M. tuberculosis* and identified a novel role for IL-15 and DAP10 in IL-22 production by NK cells. We also found that IL-22 can restrict growth of *M. tuberculosis* in macrophages by enhancing phagolysosomal fusion.

**Materials and Methods**

**Patient population**

Blood was obtained from 25 healthy tuberculin skin test negative donors. All studies were approved by the Institutional Review Board of the University of Texas Health Science Center at Tyler and informed consent was obtained from all participants.

**Abs and other reagents**

For flow cytometry, we used FITC anti-CD4, FITC anti-CD56, PE anti-CD3, PE anti-CD117, FITC anti-CD14, FITC anti-IFN-γ, (all from eBioscience), allopurinol (ICR-22R, allopurinol from IL-22 (both from R&D Systems) and FITC anti-CD8 (BD Biosciences). For neutralization, we used mAbs to IL-12, IL-15, IL-23 (all from R&D Systems) and IL-18 (MBL international). Recombinant IL-12, IL-15, IL-23 (all from R&D systems, 10 ng/ml). IL-22 (Biologend) and IL-18 (MBL international) were used for some experiments. γ-irradiated *M. tuberculosis* H37Rv was obtained from Dr. J. Belisle, Colorado State University (Fort Collins, CO) by Dr. N. Vankayalapati.
Collins, CO). GFP-expressing H37Rv was obtained from Dr. Susan Howard (University of Texas Health Science Center at Tyler, TX).

**Isolation of NK cells and monocytes**

PBMC were isolated by differential centrifugation over Ficoll-Paque (Amersham Pharmacia Biotech). Monocytes were isolated with magnetic beads conjugated to anti-CD14 (Miltenyi Biotec), and from the negative cell fraction, CD56 cells were isolated using a negative selection kit (Miltenyi Biotec), yielding cells that were 95–97% CD3 CD56. CD3–CD56+ cells were isolated by positive selection with magnetic beads conjugated to anti-CD56 (Miltenyi Biotec). The positive cells were 95–100% CD56+ and 95–97% CD3 as measured by flow cytometry. In some experiments CD3–CD56+ cells were isolated using a negative selection kit (Miltenyi Biotec), yielding cells that were 97–99% CD3–CD56+.

**Culture of NK cells and monocytes**

CD3–CD56+ cells were cultured in 24-well plates at 1 × 10^6 cells/well in RPMI 1640 containing penicillin (Life Technologies) and 10% heat-inactivated human serum, with or without 2 × 10^5 autologous monocytes/well. CD3+CD56− cells and monocytes were cultured in the presence or absence of γ-irradiated M. tuberculosis H37Rv (10 μg/ml) for 48 h at 37°C in a humidified 5% CO2 atmosphere. In some experiments, 10 μg/ml neutralizing Abs to IL-12, IL-15, IL-18, and IL-23 were added to the cultures.

**NK cell transfection and activation with IL-15**

CD3−CD56+ cells, as outlined above, were transfected with siRNA for DAP10 or scrambled small interfering RNA (siRNA) (Santa Cruz Biotechnology), using the Amaxa nucleofector (Amaxa Biosystems). In brief, 1 × 10^6 NK cells were resuspended in 100 μl of the Amaxa transfection solution and transfected with 30 pmol of siRNA, using the manufacturer’s protocol U-01. Immediately after transfection, cells were washed and transferred to 0.6 ml RPMI 1640 complete medium containing 10% heat-inactivated human serum. Approximately 25–40% of the monocytes were infected, as judged by acid-fast staining. In some experiments 1 × 10^6 IL-15-activated autologous NK cells, prepared as outlined above, were cultured in Transwell inserts (Costar) in the 12-well plates. The insert contains 0.4-μm diameter pores that allow diffusion of soluble factors but not cell-to-cell contact. In some cases, neutralizing Abs to IL-22 (10 μg/ml) were added to the Transwells.

**Measurement of intracellular mycobacterial growth**

To quantify intracellular growth of M. tuberculosis, macrophages were infected, as described above, and cultured for 7 days in RPMI 1640 and 10% human serum. After 7 days, the supernatant was aspirated, and macrophages were lysed. Bacterial suspensions in cell lysates were ultrasonically dispersed, serially diluted, and plated in triplicate on 7H10 agar. The number of colonies was counted after 3 wk. In some experiments, the number and percentage of viable macrophages after culture was assessed by Trypan blue exclusion, as described (28). In other experiments, mycobacteria in the supernatants were also plated on 7H10 agar. CFU in the bacterial lysates were always at least 10-fold greater than those in the corresponding supernatants, indicating that most organisms were intracellular.

**Confluent microscopy to evaluate phagolysosomal fusion**

Phagolysosomal fusion of infected monocytes was assessed by confluent microscopy, as described previously (29). Monocyte-derived macrophages were incubated with 0.5 μM of the acridine dye, LysoTracker Red DND-99 (Molecular Probes), washed after 2 h, and infected with GFP-expressing M. tuberculosis H37Rv, as outlined above. In some experiments, cells were cultured with IL-22. After 24 h, control and infected monocytes were fixed in 4% paraformaldehyde in phosphate buffer (pH 7.2), washed thoroughly, and mounted on Lab Tek chamber slides (Nunc).

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3 Abbreviations used in this paper: siRNA, small interfering RNA.

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**FIGURE 1.** Production of IL-22 by M. tuberculosis-stimulated CD3+CD56− NK cells. A, IL-22 production by CD3+CD56− NK cells. CD3+CD56− cells were isolated from 17 healthy donors, and cultured with autologous monocytes at a ratio of 5:1, in the presence or absence of 10 μg/ml γ-irradiated M. tuberculosis for 48 h. IL-22 concentrations were measured by ELISA. Boxes show the median and interquartile range, and whiskers show the 5th and 95th percentile values. B, IFN-γ production by CD117+ and CD117− cells. Freshly isolated CD3+CD56− cells from seven healthy donors were cultured with autologous monocytes, in the absence or presence of γ-irradiated M. tuberculosis. After 24 h, CD117+ and CD117− cells were isolated and incubated overnight in triplicate wells on an ELISPOT plate to detect IFN-γ-producing cells. Boxes show the median and interquartile range, and whiskers show the 5th and 95th percentile values. C, CD117 is not expressed by IL-22-producing NK cells. Freshly isolated CD3+CD56− cells from five healthy donors were cultured in the absence or presence of γ-irradiated M. tuberculosis and autologous monocytes. After 24 h, surface staining was performed with anti-CD117 and intracellular staining was performed with anti-IL-22. A representative figure is shown of five independent experiments.
The slides were examined using LSM 510 Meta confocal system (Carl Zeiss) equipped with an inverted microscope (Axio Observer Z1, Carl Zeiss). The infected monocytes were viewed using Plan-APochromat ×63/1.4 NA oil objective lens and ×4.3 digital magnification. An argon-krypton laser (excitation, 488 nm; emission band pass, 505–530 nm) was used to detect green fluorescence, and a helium-neon laser (excitation, 543 nm; emission limit of pass, 585 nm) to detect LysoTracker Red. Zen 2007 software (Carl Zeiss) was used for the image acquisition. The scanned images were exported and processed using Adobe Photoshop version 7.0 software (Adobe system). The percentage of GFP-H37Rv that colocalized with the LysoTracker dye was determined by counting more than 100 bacteria in at least 10 random fields. Counting was performed by an observer who did not know which slides were obtained from cultures with IL-22.

**Measurement of intracellular IL-22**

Immunolabeling to detect intracellular IL-22 was done by using the Cytofix/Cytoperm kit (BD Pharmingen). Controls for each experiment included cells that were unstained, cells to which allophycocyanin-conjugated mouse IgG had been added, and cells that had been subjected to single staining, either with the Ab to the surface marker or to the cytokine being evaluated.

**Measurement of IL-22 concentrations by ELISA**

For measurement of IL-22, supernatants from cultured cells were collected after 48 h and stored at −70°C until IL-22 concentrations were measured by ELISA (Antigenis).

**Frequency of CD117+ and CD117− cells producing IFN-γ**

CD3+ CD56− NK cells and autologous monocytes were cultured in the presence or absence of γ-irradiated *M. tuberculosis* H37Rv, as outlined above. After 24 h, CD117+ and CD117− cells were isolated, using positive selection with CD117 microbeads (Miltenyi Biotech). Aliquots of CD117+ and CD117− cells were placed on ELISPOT plates coated with anti-IFN-γ Abs (Mabtech). Following overnight incubation at 37°C in a CO2 incubator, a detection Ab was added. The plate was developed, and the number of cells that produce IFN-γ were counted. In some experiments, NK cells from freshly isolated PBMC were isolated by negative selection and CD117− cells were isolated by positive selection, and cultured in the presence or absence of γ-irradiated *M. tuberculosis* H37Rv. After 24 h, cells were placed on ELISPOT plates, and the number of cells that produced IFN-γ were counted.

**Statistical analysis**

Results are shown as the mean ± SE. For data that were normally distributed, comparisons between groups were performed by a paired or unpaired t test, as appropriate. For data that were not normally distributed, the non-parametric Mann Whitney U test was performed.

**Results**

**Freshly isolated NK cells produce IL-22 in response to *M. tuberculosis***

Freshly isolated NK cells from 17 healthy donors were cultured with autologous monocytes at a ratio of 5:1, in the presence or absence of γ-irradiated *M. tuberculosis* H37Rv. After 48 h, IL-22 levels were 8-fold-higher in the presence of *M. tuberculosis* (417 ± 99 pg/ml vs 52 ± 22 pg/ml, *p* = 0.001, Fig. 1A).

Previous studies have demonstrated that CD56<sup>bright</sup> NK cells which express CD117 (c-kit) are the major source for IFN-γ (30). To determine whether IFN-γ-producing NK cells also produce IL-22 in response to *M. tuberculosis*, we first identified NK cell subpopulation that produces IFN-γ in response to *M. tuberculosis*. NK cells obtained from healthy donors were cultured with autologous monocytes, in the presence or absence of *M. tuberculosis*. After 24 h, cells were sorted into CD117<sup>+</sup> and CD117<sup>−</sup> cells and placed on an ELISPOT plate coated with anti-IFN-γ Abs. As shown in Fig. 1B, c-kit<sup>+</sup> NK cells are the major source for IFN-γ.

To determine whether c-kit<sup>+</sup> cells produce IL-22, freshly isolated NK cells were cultured with autologous monocytes, in the presence or absence of γ-irradiated *M. tuberculosis* H37Rv. After 24 h, intracellular staining was performed to detect IL-22 in CD117<sup>+</sup> and CD117<sup>−</sup> cells. As shown in Fig. 1C, most IL-22-producing cells were CD117−. In 6 healthy donors, 2.9 ± 1% of cells were CD117<sup>+</sup> IL-22+, whereas only 0.2 ± 0.1% were CD117<sup>−</sup> IL-22+ (*p* = 0.04).

**Effect of monokines on *M. tuberculosis*-induced IL-22 production by NK cells**

Prior studies have shown that IL-23 induces IL-22 production by T cells (18, 31). To identify the monokines that induce IL-22 production by NK cells in response to *M. tuberculosis*, we isolated NK cells and monocytes from healthy donors and cultured them, with or without γ-irradiated *M. tuberculosis*, in the presence of Abs to IL-12, IL-15, IL-18, or IL-23 (all at 10 μg/ml neutralizing Abs to the cytokines shown, and supernatants were collected after 48 h. IL-22 concentrations were measured by ELISA. Control = NK cells alone, M. tb = NK cells, monocytes, and γ-irradiated *M. tuberculosis*, M. tb and anti-cytokine Ab = NK cells, monocytes, γ-irradiated *M. tuberculosis* and anti-cytokine Ab. To determine the recombinant cytokines on IL-22 production by CD3+ CD56<sup>−</sup> NK cells. Freshly isolated CD3+ CD56<sup>−</sup> NK cells from healthy donors were cultured with the recombinant cytokines shown. After 48 h, culture supernatants were collected, and IL-22 concentrations were measured by ELISA. For both panels, boxes show the median and interquartile range, and whiskers show the 5th and 95th percentile values.
Next, we determined whether IL-15 and IL-23 had additive effects on enhancing IL-22 production by NK cells. NK cells and monocytes from healthy donors were cultured, with or without γ-irradiated *M. tuberculosis*, in the presence of graded concentrations (2.5, 5, 7.5, and 10 μg/ml) of anti-IL-15 and anti-IL-23. In eight healthy donors, anti-IL-15 and anti-IL-23 did not have additive or synergistic effects in reducing IL-22 production (data not shown). This suggests that neutralization of either cytokine acts through the same pathway or mechanism to reduce IL-22 production by NK cells. The maximal inhibition was only 60% when 5 μg/ml IL-15 and IL-23 each were used (547 ± 154 pg/ml vs 206 ± 68 pg/ml).

To corroborate the results of the studies with Abs, we next determined the effect of recombinant cytokines on IL-22 production by freshly isolated NK cells, in the absence of *M. tuberculosis*. In 19 donors, IL-15 increased IL-22 levels more than 30-fold (3075 ± 676 pg/ml vs 86 ± 30 pg/ml, p < 0.001, Fig. 2B). In contrast, rIL-12 and rIL-18 had no effect. Surprisingly, rIL-23 did not increase IL-22 production (Fig. 2B).

**IL-15 induces DAP10 mRNA expression by NK cells**

The above findings suggest that IL-15 alone can induce differentiation and maintenance of IL-22-producing NK cells, whereas IL-23 requires additional signals from activated monocytes to induce IL-22 production by NK cells. We next asked whether IL-15-mediated IL-22 production depends on DAP10, an adaptor protein that is known to be involved in NK cell activation. We cultured freshly isolated NK cells, with or without IL-15 (10 ng/ml). After 48 h, DAP10 mRNA was quantified by real-time PCR, normalized to GAPDH mRNA to control for the efficiency of RNA extraction and reverse transcription in different samples. In seven healthy donors, IL-15 induced 10.6-fold DAP10 mRNA expression (p = 0.03, Fig. 3A).

**DAP10 siRNA inhibits IL-15 mediated IL-22 production by NK cells**

The above findings indicate that IL-15 increases DAP10 expression in NK cells, but does not prove that DAP10 is required for IL-22 production. To address this question, we used DAP10 siRNA to specifically inhibit DAP10-mediated signaling. Inhibition of DAP10 was confirmed by real-time PCR analysis. In eight healthy donors, DAP10 siRNA reduced IL-15-induced IL-22 production by NK cells from 1248 ± 465 pg/ml to 346 ± 120 pg/ml (p = 0.05, Fig. 3B). These findings indicate that DAP10 is required for IL-15 to induce IL-22 production by NK cells.

**IL-22 produced by NK cells inhibits growth of *M. tuberculosis* in human macrophages**

To determine whether IL-22 produced by IL-15-activated NK cells inhibits intracellular growth of *M. tuberculosis* in macrophages, CD14+ monocytes were isolated from the blood of healthy donors on day 0 and differentiated to macrophages, as outlined in the Materials and Methods. On day 3, CD3-CD56- autologous fresh NK cells were isolated and activated overnight with rIL-15. On day 4, monocyte-derived macrophages were infected with H37Rv, as outlined in the Materials and Methods. After 2 h, cells were washed to remove extracellular mycobacteria, and activated NK cells were cultured in Transwells, which were inserted into the wells containing infected macrophages. In some Transwell inserts, anti-IL-22 (IgG1) or isotype control Abs (10 μg/ml each) were added. After 7 days, Transwell inserts were removed and the number of CFU of *M. tuberculosis* H37Rv was measured, as detailed in Materials and Methods.

For six healthy donors, soluble factors produced by activated NK cells significantly reduced *M. tuberculosis* growth from 12 ± 8 × 10^6 CFU to 2.5 ± 0.34 × 10^6 CFU per well (p = 0.001; Fig. 4A). Anti-IL-22 partially abrogated this effect (6.6 ± 0.42 × 10^6 CFU, compared with 3.1 ± 0.41 × 10^6 CFU for isotype control Ab, p = 0.001, Fig. 4A).

The results above suggest that IL-22 inhibits intracellular growth of *M. tuberculosis*. To more definitively address this question, we added rIL-22 to H37Rv–infected monocyte-derived macrophages. IL-22 reduced *M. tuberculosis* CFU from 14.4 ± 2 × 10^6 to 5.2 ± 1.3 × 10^6 CFU (p = 0.02, Fig. 4B).
Effect of soluble factors produced by IL-15-activated CD3\(^+\)/H11002 phagocytes cultured with donors the mean fluorescence intensity of IL-22 receptor on macrophages further increased the level of IL-22R expression. In four healthy monocyte-derived macrophages were cultured, with or without γ-irradiated M. tuberculosis H37Rv. After 48 h, cells were stained with APC-anti-IL-22R. Control macrophages (dotted line), M. tuberculosis-cultured macrophages (thick solid line) and M. tuberculosis-cultured macrophages stained with isotype control Ab (thin solid line). Cells from four donors showed similar results, and one representative experiment is shown.

**Expression of the IL-22R by M. tuberculosis-infected macrophages**

To determine whether IL-22R is expressed on macrophages, monocyte-derived macrophages were infected with H37Rv at a MOI of 2.5:1. To some wells, autologous CD3\(^+\)/H11001 CD56\(^+\) NK cells were added at a ratio of 1 NK cell to 1 monocyte. Anti-IL-22 or mouse IgG1 isotype control Abs (10 μg/ml) were added to some wells containing NK cells in Transwells. After 7 days, the number of CFU of M. tuberculosis in each well was measured. Mean values and SEs are shown. B, rIL-22 inhibits growth of M. tuberculosis in macrophages. Monocyte-derived macrophages were infected with H37Rv at a MOI of 2.5:1. To some wells, rIL-22 (10 ng/ml), was added. After 7 days, intracellular M. tuberculosis growth in macrophages was measured. Mean values and SEs are shown for the number of CFU per well.

**FIGURE 4.** IL-22 inhibits growth of M. tuberculosis in macrophages. A. Effect of soluble factors produced by IL-15-activated CD3\(^+\)/CD56\(^+\) NK cells on intracellular growth of M. tuberculosis in monocyte-derived macrophages. Monocyte-derived macrophages were infected with H37Rv at a MOI of 2.5:1. To some wells, autologous CD3\(^+\)/CD56\(^+\) NK cells, preactivated by culture with IL-15 for 24 h, were added to Transwell inserts at a ratio of 1 NK cell to 1 monocyte. Anti-IL-22 or mouse IgG1 isotype control Abs (10 μg/ml) were added to some wells containing NK cells in Transwells. After 7 days, the number of CFU of M. tuberculosis in each well was measured. Mean values and SEs are shown. B, rIL-22 inhibits growth of M. tuberculosis in macrophages. Monocyte-derived macrophages were infected with H37Rv at a MOI of 2.5:1. To some wells, rIL-22 (10 ng/ml), was added. After 7 days, intracellular M. tuberculosis growth in macrophages was measured. Mean values and SEs are shown for the number of CFU per well.

**FIGURE 5.** Expression of the IL-22R by M. tuberculosis-stimulated macrophages. Monocyte-derived macrophages were cultured, with or without γ-irradiated M. tuberculosis H37Rv. After 48 h, cells were stained with APC-anti-IL-22R. Control macrophages (dotted line), M. tuberculosis-cultured macrophages (thick solid line) and M. tuberculosis-cultured macrophages stained with isotype control Ab (thin solid line). Cells from four donors showed similar results, and one representative experiment is shown.

**Discussion**

IL-22 was originally described as a product of activated T cells (32), particularly Th17 cells (33), and interest in this cytokine has intensified because it was found to be a critical mediator of early mucosal defense against Gram-negative bacteria that cause intestinal disease and pneumonia in mouse models (25). IL-22 is produced by murine mucosal cells that express NK cell surface markers (34, 35) and by human NK cells or NK-like cells in secondary lymphoid tissue (17, 36, 37). However, limited information is available on the role of NK cell-derived IL-22 in the human response to pathogens. In the current study, we found that human peripheral blood CD3\(^+\)/CD56\(^+\) NK cells produce IL-22 upon exposure to monocytes and M. tuberculosis, an intracellular bacterium that kills an estimated 1.9 million people annually (1, 2). IL-22 production was elicited by IL-23 and IL-15, and DAP-10, an adaptor protein, contributed to IL-15-dependent IL-22 production by NK cells. Furthermore, IL-22 inhibited intracellular mycobacterial growth by enhancing phagolysosomal fusion. Our study provides the first evidence that human NK cells can contribute to innate immunity against a major intracellular pathogen through production of IL-22.

NK cells are believed to be critical for innate defenses against viruses and intracellular bacteria, through lysis of infected cells and production of IFN-γ, which activates macrophages to kill intracellular organisms (3–5) and favors clonal expansion of Th1 cells, which are important for adaptive immune responses to eliminate these pathogens. Recent studies indicate that NK cells can also contribute to innate immunity through production of IL-22, which enhances epithelial cell proliferation and resistance to injury (22), as well as production of antimicrobial proteins (25). In the intestines of mice, commensal bacteria induce IL-22 production by cells bearing surface markers of immature NK cells (24, 35). In humans, IL-22 is produced by different NK cell subpopulations in secondary lymphoid tissue, including stage 3 CD56\(^-\)CD117\(^-\) immature NK cells (37), a subset of NKP44\(^+\)/CD56\(^-\) tonsillar NK cells (17), and fetal lymph node CD56\(^-\) NK cell precursors (36). Our current findings demonstrate that a subpopulation of peripheral blood mature CD117\(^-\) NK cells produce IL-22 but not IFN-γ.
in response to the physiologically relevant stimulus of *M. tuberculosis* in mononuclear phagocytes. This suggests that a functionally distinct NK cell subpopulation produces IL-22, consistent with findings in gastrointestinal lymphoid tissue, where IL-22-producing NK cells had limited cytolytic capacity and produced only modest amounts of IFN-γ. NK cells had limited cytolytic capacity and produced only modest amounts of IFN-γ. However, these cytokines have variable effects on growth of *M. tuberculosis*. In some experiments, cells were cultured with IL-22 (10 ng/ml). After 24 h, control and infected macrophages were fixed in 4% paraformaldehyde, washed thoroughly and mounted on Lab Tek chamber slides. Using a confocal microscope, the percentages of bacilli that were present in lysosomes were measured by an observer who had no knowledge of whether IL-22 was added to the samples. A shows the mean values and SEs of five independent experiments. B shows a representative figure.

A central feature of the pathogenicity of *M. tuberculosis* is its capacity to survive and divide in mononuclear phagocytes. Cytokines such as TNF-α and IFN-γ are crucial for protective immunity against *M. tuberculosis*, and they restrict mycobacterial proliferation in murine macrophages (38-40). However, these cytokines have variable effects on growth of *M. tuberculosis* in human macrophages, with some studies showing that IFN-γ and TNF-α enhanced bacterial replication (41, 42). We found that NK cells produced soluble factors that reduced bacillary growth in *M. tuberculosis*-infected monocyte-derived macrophages, and that this was mediated in part through IL-22. Macrophages cultured with *M. tuberculosis* up-regulated expression of IL-22 receptor 1, and signaling through this receptor resulted in increased delivery of *M. tuberculosis* to lysosomes, presumably through enhanced phagolysosomal fusion. Our findings contrast with earlier work indicating that mRNA for the IL-22 receptor 1 is not expressed by LPS-stimulated monocytes (43). This discrepancy could be due to our use of monocyte-derived macrophages, rather than monocytes, or to the effects of infection with *M. tuberculosis*. Alternatively, IL-22 receptor expression may be posttranscriptionally controlled and not reflected by changes in mRNA expression.

Previously we found that NK cells use the NKp46 and NKG2D receptors to lyse autologous *M. tuberculosis*-infected alveolar macrophages (9, 10). NK cell-derived IFN-γ can also enhance CD8+ T cell CTL activity against *M. tuberculosis*-infected target cells (44) and increase the capacity of mononuclear phagocytes to produce IL-12 and IL-18, favoring development of protective Th1 responses. Our current findings suggest that NK cells can also contribute to innate defenses against *M. tuberculosis* through production of IL-22.

IL-23 is essential for IL-22 production by T cells during infection (18, 31) and inflammatory diseases (19, 45). However, the cytokines that elicit IL-22 production by NK cells are less clearly defined. One study found that IL-15 and IL-23 were not required for murine intestinal NK cells to produce IL-22 (24), whereas another found that IL-23 stimulated IL-22 production by all splenic NK cells, and IL-22 and IL-18 elicited IL-22 production from a subpopulation of CD27high NK cells (46). In humans, IL-22 production by NK cells in lymphoid tissue was markedly increased by IL-23 (17) and IL-15 (37). We confirmed and extended these findings, demonstrating that IL-23 and IL-15, but not IL-12 or IL-18, contributed to the capacity of peripheral blood NK cells to produce IL-22 in response to *M. tuberculosis*. IL-15 alone markedly enhanced IL-22 production by NK cells, whereas IL-23 did not, suggesting that IL-23 requires additional signals from APCs to induce IL-22 production.

IL-15 is produced by activated mononuclear phagocytes, and overexpression of IL-15 enhances resistance to infection with *M. bovis* BCG infection, in part through augmenting the NK cell response (47). IL-15 is essential for development and function of NK cells, and signaling through the IL-15 receptor in NK cells requires the adaptor molecule, DAP10 (48). We found that IL-15 enhances expression of DAP10, and that IL-15-induced IL-22 production is DAP10-dependent, demonstrating an additional role for the IL-15/DAP10 signaling pathway in combating infection by intracellular pathogens.

In summary, we found that IL-15 and DAP-10 are involved in IL-22 production by NK cells in human *M. tuberculosis* infection. IL-22 inhibits intracellular growth of *M. tuberculosis* in macrophages by enhancing phagolysosomal fusion, suggesting a novel role for IL-22 in protection against intracellular mycobacteria.
Disclosures
The authors have no financial conflict of interest.

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