Interleukin 22 Inhibits Intracellular Growth of *Mycobacterium tuberculosis* by Enhancing Calgranulin A Expression

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Previously, we found that interleukin 22 (IL-22) inhibits intracellular growth of *Mycobacterium tuberculosis* in human monocyte–derived macrophages (MDMs). In the current study, we determined the mechanisms underlying these effects. We found that W7, a phagolysosomal fusion inhibitor, abrogates IL-22–dependent *M. tuberculosis* growth inhibition in MDMs, suggesting that IL-22 acts through enhanced phagolysosomal fusion. Our microarray analysis indicated that recombinant IL-22 (rIL-22) enhances the expression of an intracellular signaling molecule, calgranulin A. This was confirmed by real-time polymerase chain reaction, Western blot, and confocal microscopy. Calgranulin A small interfering RNA (siRNA) abrogated rIL-22–dependent growth inhibition of *M. tuberculosis* in MDMs. IL-22 enhanced Rab7 expression and downregulated Rab14 expression of *M. tuberculosis*–infected MDMs, and these effects were reversed by calgranulin A siRNA. These results suggest that *M. tuberculosis* growth inhibition by IL-22 depends on calgranulin A and enhanced phagolysosomal fusion, which is associated with increased Rab7 and reduced Rab14 expression.

**Keywords.** tuberculosis; Human; Cytokine; IL-22.

Recent studies have shown that proinflammatory T-helper 17 (Th17) cytokines (interleukin 17A [IL-17A], IL-17F, interleukin 21, and interleukin 22 [IL-22]) play an important role in the immune response to intracellular pathogens [1], and IL-17 is critical for vaccine-induced immunity against *Mycobacterium tuberculosis* [2, 3]. Another Th17 cytokine, IL-22, is abundant at the site of disease in patients with tuberculosis [4] and in granulomas [5]. Lung CD4+ T cells from patients with tuberculosis produce IL-22 [6], and CD4+ T cells that express membrane-bound IL-22 inhibited growth of *M. tuberculosis* in macrophages in a nonhuman primate model [7].

IL-22 is produced predominantly by CD4+ cells and shares interleukin 10R2 (IL-10R2) with other members of the IL-10 family [8, 9]. In humans, IL-22–producing CD4+ cells that express CCR6, CCR4, and CCR10 are called T-helper 22 cells [10, 11] Besides CD4+ T cells, γδ T cells, murine mucosal cells that express natural killer (NK) cell surface markers [12, 13], and human NK cells or NK-like cells in secondary lymphoid tissue also produce IL-22 [14–16]. Similar to IL-10, IL-22 enhances the survival of hepatocytes and lung epithelial cells [17–19]. IL-22 also induces the production of antimicrobial molecules and is a critical mediator of early mucosal defense against bacteria that cause intestinal disease and pneumonia in mouse models [20]. Active vitamin D enhances interleukin 6– and tumor necrosis factor α–mediated IL-22 expression, and there is increased IL-22 production in patients with psoriasis, suggesting its importance in skin homeostasis [21].

In a mouse model, we recently found that IL-22 decreases the number of immunosuppressive T-regulatory cells and contributes to the efficacy of BCG vaccination, decreasing the bacillary burden and increasing antigen-
specific T-cell responses after challenge with *M. tuberculosis* [22]. We also found that human NK cells exposed to *M. tuberculosis*–infected macrophages produce IL-22, which inhibits intracellular growth of *M. tuberculosis* [23].

In the current study, we investigated the mechanisms by which IL-22 inhibits growth of *M. tuberculosis* in human monocyte–derived macrophages (MDMs). We found that IL-22–dependent mycobacterial growth inhibition is mediated by enhancing phagolysosomal fusion; associated with increased expression of the late endosomal marker, Rab7; and requires increased expression of the calcium-binding protein, calgranulin A.

### MATERIALS AND METHODS

#### Patient Population

Blood was obtained from 15 healthy QuantiFERON-negative donors. All studies were approved by the Institutional Review Board of the University of Texas Health Science Center, Tyler, and informed consent was obtained from all participants.

#### Antibodies and Other Reagents

For flow cytometry, we used fluorescein isothiocyanate (FITC) anti-CD4, FITC anti-CD56, and FITC anti-CD14 (all from eBioscience). Human recombinant IL-22 (rIL-22; Biolegend, 10 ng/mL) was used for some experiments. γ-irradiated *M. tuberculosis* H37Rv was obtained from BEI Resources. Green fluorescent protein (GFP)–expressing H37Rv was obtained from Dr Susan Howard (University of Texas Health Science Center, Tyler). We used LysoTracker Red DND-99 (Molecular Probes); antibodies to calgranulin A, Rab7, and Rab14 (Santa Cruz Biotechnology); and W7 (N-[6-aminohexyl]-5-chloro-1-naphthalene sulfonyamide; Sigma), an inhibitor of phagolysosomal fusion.

#### Isolation of Monocytes

Peripheral blood mononuclear cells were isolated by differential centrifugation over a Ficoll-Paque gradient (Amersham Pharmacia Biotech). Monocytes were isolated with magnetic beads conjugated to anti-CD14 (Miltenyi Biotec), and positively selected cells were >95% CD14+, as measured by flow cytometry. In some experiments, the viability of MDMs was measured in 96-well plates, using the MTT assay kit (ATCC).

#### Infection of Macrophages With *M. tuberculosis* H37Rv and Measurement of Mycobacterial Growth

Monocytes (1 × 10⁶/well) were plated in 12-well plates (BD Biosciences Labware) in 1 mL of antibiotic-free Roswell Park Memorial Institute (RPMI) 1640 medium containing 10% heat-inactivated human serum. Monocytes were incubated at 37°C in a humidified 5% CO₂ atmosphere for 4 days to allow differentiation into macrophages. Some MDMs were infected with *M. tuberculosis* H37Rv at a multiplicity of infection (MOI) of 2.5:1, as described previously [24]. Cells were incubated for 2 hours at 37°C in a humidified 5% CO₂ atmosphere, washed to remove extracellular bacilli, and cultured in RPMI 1640 medium containing 10% heat-inactivated human serum. Approximately 25%–40% of the cells were infected, as judged by acid-fast staining.

Infected MDMs were cultured in RPMI 1640 medium and 10% human serum. After 3 or 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 weeks. In some experiments, the number and percentage of viable macrophages after culture was assessed by Trypan blue exclusion, as described elsewhere [25]. In other experiments, mycobacteria in the supernatants were also plated on 7H10 agar. Colony-forming units (CFU) in the bacterial lysates were always at least 10-fold greater than those in the corresponding supernatants, indicating that most organisms were intracellular.

#### Confocal Microscopy

Confocal microscopy was done to detect intracellular calgranulin A, Rab7, or Rab14 and to evaluate phagolysosomal fusion, as described previously [26]. MDMs on chamber slides (Lab Tek) were incubated with 0.5 µM of the acidotropic dye LysoTracker Red DND-99 (Molecular Probes), washed after 2 hours, and infected with GFP-expressing H37Rv at a MOI of 2.5:1 for 2 hours. They were then washed thoroughly and cultured in LysoTracker–containing complete medium with or without rIL-22. In one experiment, MDMs were not incubated with LysoTracker and were infected directly with GFP-expressing H37Rv for 2 hours, as outlined above. For intracellular staining, cells were first fixed in 2% paraformaldehyde in phosphate-buffered saline (pH 7.2) and permeabilized, and nonspecific binding was blocked by incubating in blocking buffer. Cells were then incubated overnight with mouse polyclonal antibodies to Rab7, Rab14, or calgranulin (all at 4 µg/mL) in blocking buffer. As a control, monocytes were incubated with either blocking buffer alone or secondary rabbit anti-mouse 633 antibody (4 µg/mL). Then, cells were stained with their respective secondary antibodies. The cells were washed and mounted with aqueous gel-mounting medium (Biomedia) containing antifading agent. The slides were examined using a LSM 510 Meta confocal system (Carl Zeiss) equipped with an inverted microscope (Axio Observer Z1, Carl Zeiss). The infected monocytes were viewed using a Plan-APOCHROMAT 63X/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, a helium–neon laser (excitation, 543 nm; emission limit of pass, 585 nm) was used to detect LysoTracker Red, and a helium–neon laser (excitation 633 nm) was used to detect calgranulin A, Rab7, or Rab14. Zen 2007 software (Carl Zeiss) was used for...
image acquisition. The scanned images were exported and processed using Adobe Photoshop, version 7.0, software (Adobe Systems). An observer who did not know how the cells had been treated then determined the percentage of GFP-H37Rv that colocalized with the Lysotracker dye by counting >100 bacteria in at least 10 random fields.

**Microarray Analysis**

H37Rv-infected MDMs were cultured with or without human rIL-22 for 24 hours. RNA was extracted, samples from 3–5 donors were pooled, and 2 samples from each group (H37Rv-infected MDMs and H37Rv-infected MDMs cultured with recombinant IL-22) were sent to Phalanx Biotech Group for microarray analysis.

**Small Interfering RNA (siRNA)**

MDMs were transfected with siRNA for calgranulin A or control siRNA, using transfection reagents (all from Santa Cruz Biotechnology). The efficiency of siRNA knockdown was measured by real-time polymerase chain reaction (PCR). Briefly, 10^6 MDMs were incubated with 500 µL of transfection medium and transfected with siRNA (6 pmol). After 6 hours, an additional 250 µL of 2 × RPMI complete medium was added, and cells were cultured overnight in a 24-well plate. The next day, MDMs were infected with H37Rv, as outlined above, and CFU were measured after 72 hours or confocal microscopy was performed after 48 hours.

**Real-Time PCR to Quantify Indoleamine Dioxygenase 1 (IDO1) and Calgranulin A mRNA**

Total RNA was extracted from 1 × 10^6 MDMs 24 hours after culture in different conditions, using TRIzol reagent (Life Technologies). Total RNA was reverse transcribed, using the Clone AMV First-Strand cDNA synthesis kit (Life Technologies). The forward and reverse primers for IDO1 were 5′-AGAGTCCAAATCCCCTAGTGCC3′ and 5′-AAATCAGTGCCCTCCAGTTCC-3′, respectively. Those for calgranulin A were 5′-ATTCCATGGCGCTACAGG3′ and 5′-CCTCTGGGCACTAACTCACG-3′, respectively, and primers for GAPDH were 5′-GCCATCAATGACCCCTTCCATT3′ and 5′-TTGACGCTGTCATGGAATTT-3′. Real-time PCR was performed using the Quantitect SYBR Green PCR kit (Qiagen) in a sealed 96-well microtiter plate (PE Applied Biosystems) on a spectrophotometric thermal cycler (7700 PRISM; PE Applied Biosystems). PCR analyses were performed in triplicate as follows: 95°C for 10 minutes, and 45 cycles at 95°C for 15 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. All samples were normalized to the amount of GAPDH transcript present in each sample.

**Western Blotting**

Protein was extracted from *M. tuberculosis* H37Rv-infected MDMs, cultured with or without rIL-22, and quantified by the bicinchoninic acid method (Pierce). A total of 10 µg from each sample was separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis, transferred to nitrocellulose, and probed with antibodies to calgranulin, Rab7, Rab14, or β-actin (all from Santa Cruz Biotechnology). After washing, the membranes were incubated with horseradish peroxidase–conjugated secondary antibody (Santa Cruz Biotechnology), and binding was detected by enhanced chemiluminescence (GE Healthcare).

**Statistical Analysis**

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

**RESULTS**

**Human rIL-22 Inhibits Intracellular Growth of *M. tuberculosis* Through Enhanced Phagolysosomal Fusion**

To determine whether IL-22–mediated restriction of *M. tuberculosis* H37Rv growth in macrophages depends on phagolysosomal fusion, we used W7, a chemical inhibitor of phagolysosomal fusion [27]. MDMs were generated, as outlined in Materials and Methods, and cultured with 2–120 µM of W7. After 3 days, cell viability was measured by the MTT assay and was >90% when 7.5 µM or less of W7 was added (Supplementary Figure 1). We first confirmed that 7.5 µM of W7 inhibits phagolysosomal fusion. MDMs from 5 donors were incubated with LysoTracker Red to stain lysosomes and were infected with green fluorescence protein (GFP)-expressing *M. tuberculosis* H37Rv, as described in Materials and Methods, and cultured, with or without W7. On the basis of confocal microscopy to quantify colocalization of LysoTracker Red and GFP-expressing H37Rv, W7 significantly inhibited phagolysosomal fusion (Figure 1A and 1B). This result demonstrates that W7 significantly inhibited phagolysosomal fusion during intracellular *M. tuberculosis* infection.

Next, MDMs from 4 donors were infected with *M. tuberculosis* H37Rv at a MOI of 2.5:1. To some wells, rIL-22 alone or rIL-22 and W7 were added. After 3 days, CFU in each well were measured. rIL-22 reduced the bacterial burden by 70% (*P* = .05; Figure 1C). W7 reversed the effect of rIL-22 (*P* = .05; Figure 1C), indicating that mycobacterial growth inhibition by IL-22 requires phagolysosomal fusion.

**Microarray Analysis to Identify IL-22–Regulated Genes in H37Rv-Infected MDMs**

To identify the signaling molecules that mediate IL-22–dependent *M. tuberculosis* growth inhibition, we compared gene expression of *M. tuberculosis* H37Rv–infected macrophages and rIL-22–treated infected macrophages, using microarray analysis. MDMs were infected with *M. tuberculosis* H37Rv at a
MOI of 2.5:1. To some wells, rIL-22 was added. After 24 hours, RNA was isolated, and microarray analysis was performed for 40,000 genes. mRNA expression for 39 genes was >2.0-fold higher in IL-22–treated cells (Supplementary Table 1). Among these highly expressed genes, the majority encoded chemokines and chemokine receptors. Because our focus was to identify signaling molecules that mediate IL-22–dependent inhibition of M. tuberculosis growth, we selected 2 intracellular signaling molecules, calgranulin A and IDO1, for further study. IL-22 expression positively correlates with calgranulin A expression in patients with psoriasis vulgaris [28], and IDO1 inhibits Th17 responses in M. tuberculosis infection [29]. We confirmed that IL-22 increased calgranulin A mRNA expression in M. tuberculosis H37Rv–infected MDMs 1.5-fold, using real-time PCR (Figure 2A). In contrast, IL-22 had no effect on IDO1 gene expression by M. tuberculosis H37Rv–infected MDMs (data not shown).

Expression of Calgranulin A by M. tuberculosis–Infected MDMs
To determine whether IL-22 also increased expression of calgranulin A protein, we used confocal microscopy. Infection of MDMs with M. tuberculosis H37Rv increased calgranulin A expression, and this was increased further by IL-22 (Figure 2B). Calgranulin A colocalized with phagolysosomes, and IL-22 increased delivery of M. tuberculosis H37Rv to LysoTracker Red + lysosomes (Figure 2B); and the percentage of bacilli that were in lysosomes increased from 20% ± 4% to 61% ± 4% (P = .0002; Figure 2C).

IL-22–Dependent Inhibition of M. tuberculosis Growth Depends on Calgranulin A
To determine whether calgranulin A is required for IL-22–mediated inhibition of M. tuberculosis H37Rv growth, we inhibited calgranulin A expression with siRNA. MDMs were transfected with calgranulin A or scrambled siRNA, infected with M. tuberculosis H37Rv, and cultured in the presence or absence of rIL-22. In 6 healthy donors, calgranulin A siRNA completely abrogated the inhibitory effect of IL-22 on mycobacterial growth (P = .003; Figure 3), whereas scrambled siRNA had no effect. These findings demonstrate that IL-22–mediated inhibition of M. tuberculosis H37Rv growth in MDMs is dependent on calgranulin A.

Figure 1. Effect of W7 on phagolysosomal fusion and on interleukin 22 (IL-22)–mediated inhibition of Mycobacterium tuberculosis in human monocyte–derived macrophages (MDMs). A, Phagolysosomal fusion in H37Rv-infected MDMs. MDMs from 3 healthy donors were incubated with 0.5 µM of the acidotropic dye, LysoTracker Red DND-99, for 1 hour, washed, and infected with M. tuberculosis green fluorescent protein (GFP) H37Rv at a multiplicity of infection (MOI) of 2.5:1. After 2 hours, wells were washed, and some were incubated with W7, a chemical inhibitor of phagolysosomal fusion. Cells were fixed after 1 hour and analyzed by confocal microscopy. B, Mean values and standard errors (SEs) are shown for the percentages of GFP-expressing bacilli that colocalized with LysoTracker Red. C, Growth of M. tuberculosis H37Rv. MDMs from 4 healthy donors were infected with M. tuberculosis H37Rv at a MOI of 2.5:1. To some wells, recombinant IL-22 (rIL-22) alone or rIL-22 and W7 were added. After 3 days, the number of colony-forming units (CFU) in each well were measured. Mean values and SEs are shown.
Calgranulin A Enhances IL-22–Induced Phagolysosomal Fusion

We next sought to determine whether calgranulin A contributes to IL-22–mediated phagosomal maturation. MDMs from 4 donors were transfected with calgranulin A or scrambled siRNA, infected with M. tuberculosis H37Rv, and cultured in the presence or absence of rIL-22. IL-22 increased colocalization of M. tuberculosis and lysosomes, but this effect was completely negated by calgranulin A siRNA (Figure 4A), which reduced colocalization by 75% ($P = .003$; Figure 4B).

IL-22 Affects Expression of Rab7 and Rab14 Through Calgranulin A

Phagosomal maturation is characterized by the sequential recruitment of Rab proteins, including the late endosomal marker, Rab7 [30], whereas expression of Rab14 is associated with arrest of phagosomal maturation [31]. M. tuberculosis prevents phagosomal maturation by preventing acquisition of Rab7 [32–34] and by increasing expression of Rab14 [31, 33]. To identify the stages of phagolysosomal fusion that are affected by IL-22 and calgranulin A, we first infected MDMs with M. tuberculosis H37Rv at a MOI of 2.5:1 and cultured them with or without rIL-22. After 10, 30, and 180 minutes, calgranulin A, Rab7, and Rab14 protein expression were determined by Western blot. rIL-22 enhanced calgranulin A expression by M. tuberculosis–infected macrophages within 10 minutes (Figure 5A and 5B) and enhanced Rab7 expression within 30 minutes (Figure 5A and 5C). In contrast, rIL-22 inhibited Rab14 expression by M. tuberculosis H37Rv–infected macrophages within 30 minutes (Figure 5A and 5D).
Next, MDMs from 4 donors were infected with GFP-expressing *M. tuberculosis* H37Rv at a MOI of 2.5:1 for 30 minutes and then cultured with or without IL-22. After 2 hours, macrophages were stained with anti-Rab7 and analyzed by confocal microscopy. rIL-22 enhanced Rab7 expression and increased the percentage of *M. tuberculosis* H37Rv that colocalized with Rab7 (38% ± 5% vs 14% ± 3%; *P* = .005; Figure 6A and 6B). Calgranulin A siRNA inhibited IL-22–dependent colocalization of H37Rv and Rab7, compared with control siRNA (19% ± 2% vs 30% ± 5%; Figure 6A and 6B), suggesting that calgranulin A is necessary for IL-22–mediated expression of Rab7 on the phagosome.

We also evaluated the effects of IL-22 and calgranulin A on expression of Rab14. MDMs from 4 healthy donors were infected with GFP-expressing *M. tuberculosis* H37Rv at a MOI of 2.5:1 for 30 minutes and then cultured with or without IL-22. After 30 minutes, macrophages were stained with anti-Rab14 and analyzed by confocal microscopy. Rab14 expression was markedly increased by infection with *M. tuberculosis* H37Rv and reduced by IL-22 (Figure 6C). IL-22 reduced colocalization of *M. tuberculosis* H37Rv and Rab14 (59% ± 4% vs 27% ± 6%; *P* = .01; Figure 6C and 6D), but this effect was reversed by calgranulin A siRNA (50% ± 4% vs 36% ± 5%; *P* = .02; Figure 6C and 6D). These findings suggest that IL-22 and calgranulin A downregulate Rab14 expression on the phagosome, reversing the effects of infection with *M. tuberculosis*.

**DISCUSSION**

IL-22 plays an important role in the mucosal defense against bacteria that cause intestinal disease and pneumonia. We...
recently showed that activated human NK cells produce IL-22, which reduces intracellular growth of *M. tuberculosis* in macrophages [23], providing the first evidence that a soluble product of NK cells exhibits antimycobacterial activity. In this report, we demonstrate that IL-22 acts by enhancing phagosomal maturation, because treatment of *M. tuberculosis*–infected macrophages with W7, a phagolysosomal fusion inhibitor, abolished IL-22–dependent inhibition of *M. tuberculosis* growth. *M. tuberculosis* delays phagosomal maturation by preventing acquisition of Rab7 and recruiting Rab14 to the phagosome [30, 31, 35, 36]. These effects were reversed by IL-22, and the capacity of IL-22 to facilitate phagosomal maturation and alter Rab expression was mediated through upregulation of calgranulin A. Thus, our study identifies calgranulin A as a central molecule through which IL-22 inhibits intracellular growth of *M. tuberculosis*.

IL-22 is produced by activated T cells [37], particularly Th17 cells [38], and is a critical mediator of early mucosal defense against bacteria that cause intestinal disease and pneumonia in mouse models [20]. Although mice in which the IL-22 gene was deleted do not show increased susceptibility to tuberculosis [39], studies in humans and nonhuman primates suggest that IL-22 contributes to antimycobacterial immunity, because IL-22–producing *M. tuberculosis*–responsive CD4+ T cells are present in persons with latent *M. tuberculosis* infection [6], IL-22 levels are elevated at the site of disease in patients with tuberculosis [4], and CD4+ T cells from *M. tuberculosis*–infected nonhuman primates produce IL-22, which inhibits growth of *M. tuberculosis* in macrophages [7, 23]. Furthermore, we found that human NK cells exposed to *M. tuberculosis*–infected macrophages produce IL-22, which inhibits intracellular growth of *M. tuberculosis* [23]. However, the mechanisms through which

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**Figure 5.** Expression of calgranulin A, Rab7, and Rab14 proteins at different time points in interleukin 22 (IL-22)–treated *Mycobacterium tuberculosis*–infected human monocyte–derived macrophages (MDMs). MDMs from 3 healthy donors were infected with *M. tuberculosis* at a multiplicity of infection of 2.5:1 and were or were not treated with recombinant IL-22 (rIL-22). After 10, 30, and 180 minutes, whole-cell lysates were prepared. Lysates were subjected to Western blotting with an antibody to calgranulin A, Rab7, and Rab14. The blot was stripped and reprobed with an antibody to a β-actin. The result is representative of experiments performed in 3 donors. All protein bands were quantified. Mean values and standard errors are shown. A, A representative Western blot figure is shown. B, Expression of calgranulin A in 3 donors. C, Expression of Rab7 in 3 donors. D, Expression of Rab14 in 3 donors.
IL-22 inhibits *M. tuberculosis* growth in macrophages remain uncertain. Alveolar macrophages are one of the first cells to encounter *M. tuberculosis* after infection, and macrophages are critical for innate defenses against *M. tuberculosis*, through phagocytosis of the bacilli, followed by a complex process of phagosomal maturation that results in fusion with lysosomes and mediates bacterial killing. Rab GTPases play a pivotal role in phagosomal...
molecular partners to increase expression of Rab7 and reduce the recruitment and activation of early endosome antigen 1 [42]. After its activation, Rab5 dissociates, and Rab7 is recruited to the phagosome through a process that requires phosphatidylinositol 3-phosphate [33, 42–44]. *M. tuberculosis* is a consummate intracellular pathogen and remains viable in macrophages by arresting phagosomal maturation, preventing the acquisition of Rab7 and Rab7-mediated cathepsin D to the phagosomal membrane through production of a secreted acid phosphatase that hydrolyses phosphatidylinositol 3-phosphate [34, 42]. *M. tuberculosis* also increases Rab14 expression on phagosomes, which stimulates fusion with early endosomes, rather than late endosomes, preventing phagosomal maturation to lysosomes [31]. In the current report, we found that IL-22 recruited more Rab7 and less Rab14 to phagosomes, reversing the delayed maturation induced by *M. tuberculosis*. Both of these effects were mediated through IL-22-dependent upregulation of calgranulin A.

Early studies suggested that IL-22 should not affect macrophages because the IL-22 receptor (IL-22R) was only present on human epithelial cells, not hematopoietic cells [19, 45]. More recent studies have found that, during *M. tuberculosis* infection, macrophages from humans and nonhuman primates express IL-22R [7, 23]. Our microarray data indicate that IL-22 induces expression of approximately 40 genes in *M. tuberculosis*-infected macrophages, including the gene encoding calgranulin A. Addition of IL-22 upregulated expression of calgranulin A in *M. tuberculosis*-infected macrophages, at the levels of both mRNA and protein, as documented by real-time PCR analysis and confocal microscopy. Our findings are consistent with findings that IL-22 stimulates expression of calgranulin A in human keratinocytes and that IL-22 expression positively correlates with calgranulin A expression in patients with psoriasis vulgaris [28, 46].

Calgranulin A is a member of the S100 family of calcium-binding proteins. We speculate that IL-22 increases intracellular Ca\(^{2+}\) flux, which is known to eliciting changes that enhance phagolysosomal fusion, through upregulation of calmodulin and sphingosine kinase activity [47, 48]. In neutrophils, increased intracellular Ca\(^{2+}\) concentrations result in recruitment of calgranulin A to the phagosomal membrane and increased expression of phagosomal reactive oxygen species [49]. We hypothesize that IL-22 increases intracellular Ca\(^{2+}\), resulting in trafficking of calgranulin A to the phagosomal membrane. Like other S100 proteins, calgranulin A consists of 2 EF-hand regions that bind Ca\(^{2+}\), linked by a hinge region. Binding of Ca\(^{2+}\) causes a conformational change that opens the molecule, exposing a wide hydrophobic cleft in the hinge region, which can then interact with a target protein [50]. Calgranulin A could then interact with as yet unidentified phagosome-associated molecular partners to increase expression of Rab7 and reduce expression of Rab14, thereby increasing phagosomal maturation and increasing mycobacterial killing.

In summary, our study delineates a mechanism through which NK cells can inhibit intracellular growth of *M. tuberculosis* through IL-22 production. We found that IL-22 upregulates expression of calgranulin A, which in turn favored phagosomal maturation, enhancing expression of Rab7 and decreasing expression of Rab14 on *M. tuberculosis*-containing phagosomes.

**Supplementary Data**

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

**Financial support.** This work was supported by the National Institutes of Health (grants AI054629, AI06366, and A1085135 to R. D. and the Potts Memorial Foundation grant to R. D.), the Cain Foundation for Infectious Disease Research, and the Center for Pulmonary and Infectious Disease Control.

**Potential conflicts of interest.** All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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