Evidence against a Role for Interleukin-10 in the Regulation of Growth of Mycobacterium avium in Human Monocytes

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Interleukin-10 (IL-10) inhibits intracellular Mycobacterium avium killing by cytokine-activated murine macrophages and may have a role in pathogenesis. Cytokine activities in supernatants of M. avium–infected human monocytes were maximal at 6–24 h for tumor necrosis factor (TNF)-α and 24–48 h for IL-10. TNF-α and IL-10 production increased with increasing M. avium–to–monocyte infection ratios (20:1 to 200:1). TNF-α production by monocytes infected with smooth, domed, and opaque organisms at 200:1 exceeded that of monocytes infected with smooth, flat, and transparent M. avium (P < .01). IL-10 induction demonstrated considerable strain-to-strain variability and did not correlate with intracellular M. avium growth. IL-10 significantly inhibited TNF-α, IL-1β, and IL-6 production by M. avium–infected monocytes. Coculturing monocytes with IL-10 after M. avium infection did not affect intracellular M. avium growth. Differential induction of TNF-α may be a factor in the intracellular growth of M. avium in human monocytes. IL-10, however, played no apparent role in pathogenicity in this model.

Disseminated Mycobacterium avium infection is the most frequent bacterial infection in AIDS patients [1–3]. Mononuclear phagocytes are critical effector cells in host defenses against intracellular pathogens such as mycobacteria. The main factor that has been identified that determines the intracellular growth of M. avium in human monocytes is colonial morphology. The smooth, flat, and transparent (SmT) morphotype is more virulent in vivo and associated with increased capacity for intracellular growth compared with the smooth, domed, and opaque (SmD) morphotype [4]. M. avium infection of human monocytes induces interleukin (IL)-1α, IL-1β, IL-6, and tumor necrosis factor (TNF)-α expression [5, 6]. The SmD (avirulent) M. avium morphotype induces more IL-1α, IL-1β, and TNF-α, but not IL-6, release into culture supernatants at 24 h than does the SmT morphotype. IL-1α and IL-6 promote the intracellular and extracellular growth of M. avium [7, 8], whereas TNF-α, interferon (IFN)-γ, and granulocyte-macrophage colony-stimulating factor (GM-CSF) activate macrophages to inhibit intracellular M. avium growth [4, 9, 10]. The balance between these mycobacterial growth-enhancing and -inhibiting cytokines may be important in regulating monocyte effector functions against M. avium infection.

IL-10 is an immunosuppressive cytokine produced by Th2 cells and macrophages that inhibits IFN-γ production and antigen-specific T cell proliferation by diminishing antigen-presenting cell function [11, 12]. IL-10 inhibits cytokine production (e.g., IL-1, IL-6, IL-8, TNF-α, GM-CSF, and macrophage-CSF [M-CSF]) by activated human monocytes/macrophages [13–16]. IL-10 also inhibits the effector function of murine macrophages activated by IFN-γ or GM-CSF, enhancing intracellular M. avium growth [17]. Moreover, coculture with anti-IL-10 antibody decreases in vitro intracellular M. avium growth in murine macrophages [17], and intraperitoneal infusion of mice with anti-IL-10 antibody enhances resistance against M. avium infection in vivo [18].

In this study, we analyzed the effect of IL-10 on intracellular growth of M. avium using a human monocyte infection model. We also examined the relationships between intracellular M. avium growth, TNF-α and IL-10 expression, and the modulation of expression of other cytokines by IL-10.

Materials and Methods

Microorganisms. M. avium LR114 and LR147 were donated by J. F. Crawford (CDC, Atlanta). LR114 is a non–AIDS-associated serovar 4 isolate that contains no plasmids. The cloned strains LR114F (SmT morphotype) and LR114R (SmD morphotype) were subcultured from the parent strain on the basis of colonial morphology. LR114F has flat and transparent morphology and was highly pathogenic (high capacity for intracellular growth) compared with LR114R which has domed, opaque, colonial morphology and is nonpathogenic [4, 19]. LR147, a non–AIDS-associated isolate, is another highly pathogenic strain exhibiting flat colony morphology. M. avium 86m2096 (flat and rough colonial morphology) is a clinical isolate from an AIDS patient with disseminated M. avium infection treated at University Hospitals, Cleveland. Strains 2-151SmT and 2-151SmD were donated by I. Orme (Colorado State University, Fort Collins).

All M. avium strains were grown in Middlebrook 7H9 broth (Difco, Detroit) supplemented with albumin-dextrose-catalase and incubated at 37°C in 5% CO2 in air. When the bacterial density
was \( \sim 1 - 5 \times 10^9/\text{mL} \) (log-phase growth), the mycobacteria were aliquoted into cryotubes and stored frozen at \(-70^\circ C\) until use. Colonial morphology of each stock was checked on 7H10 agar, and conversion of SmT to SmD and reversion of SmD to SmT were not seen in the stock used. Before infection, bacteria were thawed and uniformly resuspended by sonication for 20 s using a high-intensity ultrasonic processor (Vibra Cell; Sonic & Materials, Danbury, CT) to obtain a single-cell suspension and diluted with RPMI 1640 (Whittaker Bioproducts, Walkersville, MD) containing 5% autologous unheated serum.

**Monocyte isolation.** Peripheral blood mononuclear cells (PBMC) from healthy volunteers were separated by the ficoll-hypaque sedimentation method from heparinized fresh venous blood. PBMC were suspended at 10^7/mL in RPMI 1640, and 5 mL was added to plastic petri dishes (Falcon 3003; Becton Dickinson Labware, Lincoln Park, NJ) precoated with 1.5 mL of pooled heat-inactivated human serum. After 60 min of incubation at 37°C, the dishes were gently shaken and nonadherent cells were removed. The dishes then were washed twice with warmed RPMI 1640 supplemented with 10% (vol/vol) heat-inactivated fetal calf serum. Adherent cells were removed by scraping with a rubber policeman after 20 min of incubation at 4°C in cold PBS without calcium and magnesium. The adherent cells were 90%-95% monocytes, as determined by peroxidase cytochemistry.

**Generation of cytokines by human monocytes infected with **\( M. avium \). Adherent cells were suspended at 10^6 cells/mL in RPMI 1640 containing 5% unheated autologous serum. The cell suspension was placed into 6-ml polystyrene, round-bottom tubes (1 mL/tube). Monocytes were infected with 1 of 6 \( M. avium \) strains at infection ratios from 10:1 to 300:1 (\( M. avium \) to–monocyte ratio) for 60 min at 37°C. The cultures then were centrifuged at 100 g for 5 min; supernatants containing extracellular bacteria were aspirated and discarded. The cells were washed once with prewarmed RPMI 1640. Monocytes were resuspended in Iscove's modified Dulbecco's medium (IMDM) containing 1% autologous unheated serum and incubated with 10% (vol/vol) heat-inactivated human serum. After 60 min of incubation at 37°C, the plates were washed twice with warmed RPMI 1640 supplemented with 10% (vol/vol) heat-inactivated fetal calf serum. Adherent cells were removed by scraping with a rubber policeman after 20 min of incubation at 4°C in cold PBS without calcium and magnesium. The adherent cells were 90%-95% monocytes, as determined by peroxidase cytochemistry.

**Quantification of TNF-\( \alpha \), IL-1\( \beta \), and IL-6 in monocyte culture supernatants by ELISA.** Immunologically reactive TNF-\( \alpha \), IL-1\( \beta \), and IL-6 were measured in monocyte supernatants by the sandwich ELISA method. Immulon 4 (Dynatech, Chantilly, VA) ELISA plates were coated with mouse anti-human TNF-\( \alpha \) monoclonal antibody (Mab) (Endogen, Boston), or anti-human IL-1\( \beta \) or IL-6 MAb (R & D Systems, Minneapolis) and incubated at 4°C overnight. The plates were washed three times with 0.1% bovine serum albumin (BSA)-PBS (washing buffer) and then blocked with 1% BSA-PBS for 60 min at 37°C. Samples (if necessary) and standards were diluted with 1% BSA-PBS. Aliquots (100 \( \mu L \)) of samples and standards were added to test wells and incubated at 37°C for 60 min (TNF-\( \alpha \)) or at room temperature for 2 h (IL-1\( \beta \) and IL-6). After the plate was washed three times with washing buffer, rabbit anti-human TNF-\( \alpha \) antibody (Genzyme Diagnostics, Cambridge, MA) diluted 1/1000 with 1% BSA-PBS, goat anti-human IL-1\( \beta \), or IL-6 antibody (R & D Systems) diluted 1/2000 with 1% BSA-PBS was added to each well. The plates were then incubated for 1 h at 37°C (TNF-\( \alpha \)) or 2 h at 22°C (IL-1\( \beta \) and IL-6). Rabbit or goat IgG bound in each well was detected by goat anti-rabbit IgG peroxidase-conjugated or rabbit anti-goat IgG peroxidase-conjugated antibody (Sigma, St. Louis) diluted 1/5000 with 1% BSA-PBS, respectively. The plates were washed four times with washing buffer, and peroxidase substrate (o-phenylene-diamine dihydrochloride) was added to each well to determine peroxidase activity. The color intensity was measured as absorbance (optical density) at 490 nm using an automated ELISA plate reader (Molecular Devices, Menlo Park, CA). The sensitivity of the assay for detection of each cytokine was 10 pg/mL.

**Expression of cytokine mRNA.** Adherent monocytes were infected with \( M. avium \) (SmT or SmD) and cultured in 10-mm petri dishes (Falcon 3003) for 18 h. Total RNA was extracted from monocytes by the TRI REAGENT method (Life Technologies GIBCO BRL, Gaithersburg, MD). After the culture supernatant was removed, monocyte monolayers were lysed by 0.5 mL of TRI REAGENT; 100 \( \mu L \) of chloroform was added to each sample. Resulting mixtures stood at room temperature for 15 min and were then centrifuged at 12,000 \( g \) for 15 min at 4°C. The aqueous phase was transferred to a fresh tube, and RNA was precipitated with isopropanol. IL-10–specific mRNA was determined by the reverse transcriptions–polymerase chain reaction (RT-PCR) technique.[20]

The following primers were used for IL-10 and hypoxanthine phosphoribosyl transferase (HPRT) in the PCR: IL-10, CTGAGAACCCAAGCCAGACATCAAG (5' primer); IL-10, CAAATAGGTTCATCAGGCGTGGC (3' primer); HPRT, CGAATGTGATGAAGGAGATGG (5' primer); HPRT, GGATCTATACTGCCTGACCAAGG (3' primer). RNA (1 \( \mu g \)) was mixed with 1.25 \( \mu L \) of 2.5 mM dNTPs (Boehringer Mannheim Biochemicals, Indianapolis), 5 \( \mu L \) of 5x reverse transcriptase buffer, 2.0 \( \mu L \) of 1 mM dithiothreitol, 0.5 \( \mu L \) of RNasin (40,000 U/mL), 1.0 \( \mu L \) of reverse transcriptase (200 U/mL), and 4.0 \( \mu L \) of 25 mM MgCl\(_2\) and reverse-transcribed using 2 \( \mu L \) of random hexamer primers (50,000 \( A_{\text{so}} \) mM/\( \mu L \)); Boehringer Mannheim). PCR mixtures containing 2 \( \mu L \) of 25 mM MgCl\(_2\), 5 \( \mu L \) of 10x PCR buffer, 5 \( \mu L \) of 10 mM 5' primer, 5 \( \mu L \) of 10 mM 3' primer, and 1 U of Taq polymerase (Promega, Madison, WI) in a final volume of 40 \( \mu L \) were prepared. PCR was done in an Omniteng thermocycler (Hybaid, Holbrook, NY) as follows: IL-10, 1 cycle of 30 s at 94°C, 35 cycles of 10 s at 94°C, 1 min at 70°C, and 2
cultured monocytes were removed; a lysing solution containing
0 (immediately after infection) and 4 and 7 days after infection.

at each time point was determined using a cfu assay [4, 7, 20].

Immediately and 4 and 7 days after infection, supernatants of
monocytes were infected with LR114SmT (smooth, flat, and transparent)
(○) or LR114SmD (smooth, domed, and opaque) (●) strains for 60 min or stimulated with 10 μg/mL lipopolysaccharide (LPS) (△) and then cultured in Iscove’s modified Dulbecco’s medium containing 1% autologous serum for up to 7 days. Samples were harvested 6, 24, 48, 96, and 168 h after infection. At each time point, a representative culture was harvested. IL-10 concentrations in culture supernatants were determined by ELISA. Data shown are mean of 3 independent experiments using monocytes from 3 subjects.

Effect of IL-10 on intracellular M. avium growth in human monocytes. PBMC suspension (50 μL/well; 10⁷ cells/mL) in RPMI 1640 supplemented with 5% autologous unheated serum was used for infection. Monocytes infected with M. avium were cultured with 1% autologous unheated serum. The plates were incubated at 37°C for 60 min to allow monocytes to adhere firmly to the wells. Nonadherent cells were gently removed by washing twice with warm RPMI 1640. After the final wash, each well contained 2–5 × 10⁵ monocytes as assessed by nuclear counting using naphthol blue-black dye [21]. Cell detachment after 7 days of culture was <20%. Monocytes were infected with M. avium by replacing the medium with mycobacterial suspension in RPMI 1640 supplemented with 5% autologous unheated serum. The plates were incubated at 37°C for 60 min, and each well was gently washed three times with warmed medium to remove extracellular M. avium. After infection, monocytes were cultured in the presence or absence of recombinant human IL-10 (final concentration, 0–20 ng/mL) in RPMI 1640 supplemented with 5% autologous serum for up to 7 days. Samples were harvested at day 0 (immediately after infection) and 4 and 7 days after infection. In some experiments, monocytes were pretreated with IL-10 for 2 days prior to infection.

cfu assay. The number of living M. avium in monocyte lysates at each time point was determined using a cfu assay [4, 7, 20]. Immediately and 4 and 7 days after infection, supernatants of cultured monocytes were removed; a lysing solution containing

<110 μL of 7H9 medium and 40 μL of 0.25% SDS in PBS was added to each well. The plates were incubated at room temperature for 10 min. The lysates then were transferred to tubes containing 50 μL of 20% bovine serum albumin to neutralize the SDS. Cell lysates were sonicated for 20 s and serially 10-fold–diluted in 7H9 broth. Three 10-μL aliquots of each dilution were plated on Middlebrook 7H10 agar (Difco) in 60-mm-diameter petri dishes (Falcon no. 1007). The spots were allowed to absorb onto the surface of the 7H10 plates at room temperature to prevent them from running together. The agar plates were incubated for 4–6 days at 37°C in a humidified atmosphere with 5% CO₂ until bacterial colonies were visible. The number of colonies in each of the three spots for each dilution was counted with a stereomicroscope and averaged. The results were expressed as mean ± SE of cfu per milliliter of cell lysate, which represented the bacterial cfu associated with ~10⁵ monocytes.

Statistical analysis. The significance of differences between groups was calculated by Student’s t test or paired t test.

Results

IL-10 and TNF-α expression by human monocytes infected with M. avium. The kinetics of IL-10 expression by human monocytes infected with M. avium were examined. Peripheral blood monocytes from healthy donors were infected with isogenic SmT or SmD morphotypes of M. avium (LR114) and cultured in IMDM containing 1% autologous unheated serum for up to 7 days. Culture supernatants were harvested 6, 24, 48, 96, and 168 h after infection, and the levels of IL-10 and TNF-α in culture supernatants were determined by ELISA. TNF-α levels increased rapidly after M. avium infection, peaking at 6–24 h and declining thereafter, as reported previously [5]. Minimal IL-10 activity was detected at early time points
Figure 2. Relationship between M. avium burdens and IL-10 production by M. avium–infected monocytes. Monocytes were infected with LRI14SmT (SmT = smooth, flat, and transparent) (●), LRI14SmD (○), 2-151SmT (△), 2-151SmD (SmD = smooth, domed, and opaque) (▲), 86m2096 (□), or LR147 (■) strains (4 pathogenic and 2 nonpathogenic strains) at infection ratios (M. avium to monocyte) from 10:1 to 300:1. Culture supernatants were harvested at 24 h. IL-10 activity was determined by ELISA. No. of M. avium in monocyte lysates was assessed by cfu assay. Data are from representative experiment; SE for IL-10 concentration and cfu was <10%. Similar results were obtained using monocytes from another subject.

(3- and 6-h culture supernatants); however, IL-10 production increased rapidly after 6 h of stimulation and peaked at 24–48 h in monocyte cultures infected with M. avium. IL-10 production in LPS-stimulated cultures peaked at 48 h (figure 1).

The relationship of M. avium dose in monocyte lysates and IL-10 production was then examined. Three SmT (LRI14SmT, 2-151SmT and LR147), 1 flat and rough (86m2096), and 2 SmD (LR144SmD and 2-151SmD) morphotypes of M. avium strains were used. Monocytes from healthy participants were infected with 1 of 6 strains of M. avium at M. avium–to-monocyte infection ratios of 10:1 to 300:1. Culture supernatants were harvested at 24 h, the time of maximal TNF-α and IL-10 expression (as determined earlier). Monocytes were lysed, and the number of M. avium in monocyte lysates was assessed by cfu assay. IL-10 activity in 24 h culture supernatants increased with increasing M. avium number ingested by monocytes (figure 2). Significant strain-to-strain variation was observed in IL-10 production by M. avium–infected monocytes. There was no correlation between IL-10 release into culture supernatants and M. avium colonial morphology.

TNF-α production by monocytes infected with SmT or SmD strains of M. avium at different infection ratios was examined next. TNF-α was undetectable (<10 pg/mL) in supernatants of uninfected monocytes. TNF-α levels in culture supernatants of monocytes infected with SmD strains (11.5 ± 1.8 ng/mL, n = 6) were significantly higher than in those of monocytes infected with an isogeneic SmT strain (5.0 ± 1.7 ng/mL, n = 6) at a 200:1 infection ratio (P < .05, paired t test). TNF-α levels in supernatants of monocytes infected with SmD organisms at a 200:1 infection ratio were 16.8 ± 3.4-fold higher than in monocyte supernatants infected at a 20:1 infection ratio; the comparable ratio for 200:1/20:1 organisms was only 9.6 ± 3.2-fold for the SmT strain (P < .02, Student’s t test). The number of M. avium in monocyte lysates infected with SmT and SmD morphotypes was comparable at the same infection ratio (data not shown).

IL-10 and TNF-α production by monocytes infected with various strains of M. avium. Table 1 shows TNF-α and IL-10 production by monocytes obtained from 12 healthy donors after infection with 1 of 6 M. avium strains. LPS induced greater TNF-α production than did M. avium infection. SmD M. avium strains induced higher levels of TNF-α production than did isogeneic SmT morphotypes (P < .01 for LR114 and P < .05 for 2-151, paired t test). However, there were no significant differences in IL-10 concentrations of culture supernatants between monocytes infected with SmT and SmD morphotypes of M. avium strains. TNF-α concentrations in supernatants from monocytes infected with strain 86m2096, an AIDS-associated clinical isolate with flat and rough colonial morphology, were intermediate between those seen after infection with other SmT and SmD strains. Strain 86m2096 induced IL-10 production comparable to that of other SmT and SmD strains. Infection of monocytes with the highly pathogenic strain LR147 (SmT morphotype) induced significantly lower levels of TNF-α than did infections with the 114SmD and 2-151SmD strains (P < .05, Student’s t test).

Table 1. Tumor necrosis factor (TNF)-α and IL-10 production by human monocytes infected with various strains of M. avium.

<table>
<thead>
<tr>
<th>Strains</th>
<th>n</th>
<th>TNF-α (ng/mL)</th>
<th>IL-10 (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>10 µg/mL LPS</td>
<td>12</td>
<td>18.6 ± 3.8</td>
<td>3.3 ± 0.5</td>
</tr>
<tr>
<td>Strains</td>
<td>n</td>
<td>TNF-α (ng/mL)</td>
<td>IL-10 (ng/mL)</td>
</tr>
<tr>
<td>LRI14SmT</td>
<td>12</td>
<td>3.7 ± 1.2*</td>
<td>2.1 ± 0.3</td>
</tr>
<tr>
<td>LRI14SmD</td>
<td>12</td>
<td>8.4 ± 2.0*</td>
<td>2.8 ± 0.4</td>
</tr>
<tr>
<td>2-151SmT</td>
<td>6</td>
<td>2.5 ± 1.2</td>
<td>1.3 ± 0.4</td>
</tr>
<tr>
<td>2-151SmD</td>
<td>6</td>
<td>7.4 ± 2.4*</td>
<td>2.0 ± 0.5</td>
</tr>
<tr>
<td>86m2096</td>
<td>9</td>
<td>6.2 ± 2.3</td>
<td>2.6 ± 0.5</td>
</tr>
<tr>
<td>LR147 (SmT)</td>
<td>5</td>
<td>1.1 ± 0.5</td>
<td>1.2 ± 0.4</td>
</tr>
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</table>

NOTE. Data are mean ± SE. Monocytes were infected with SmT or SmD morphotypes at an infection ratio (M. avium to monocytes) of 50:1. Culture supernatants were harvested at 24 h; IL-10 and TNF-α concentrations were determined by ELISA. LPS, lipopolysaccharide; SmT, smooth, flat, and transparent; SmD, smooth, domed, and opaque.

* P < .01, † P < .05, paired t test.
**IL-10-specific mRNA expression by M. avium--infected monocytes.** Next, IL-10--specific mRNA expression of monocytes infected with various strains of M. avium was examined. Monocyte monolayers (5–10 × 10^6 cells/petri dish) were infected by incubation with 1 of 5 M. avium strains (25 × 10^7 bacteria in 5 mL of complete medium) for 60 min and then washed and cultured for 24 h in IMDM with 1% autologous serum. Total RNA was extracted from monocyte monolayers, and IL-10--specific mRNA was analyzed by RT-PCR and Southern blot. Figure 3 shows IL-10- and HPRT-specific mRNA by RT-PCR products and Southern blot from two independent experiments. Monocytes infected with LR114SmD and 86m2096 expressed more IL-10 mRNA than did monocytes infected with LR114SmT or 2-151SmT. Expression of mRNA by M. avium--infected monocytes paralleled IL-10 production in culture supernatants determined by ELISA for these strains.

**Up-regulation of IL-10 production by TNF-α.** TNF-α regulates IL-10 expression by induction or augmentation (or both) of IL-10 production by human monocytes [22]. We examined whether anti-TNF-α antibody modulated endogenous IL-10 release after infection of monocytes with M. avium. In the presence of 10 μg/mL goat anti-human TNF-α antibody, IL-10 production by M. avium--infected monocytes was inhibited 20%–70% compared with that of monocytes cultured with control antibody (goat IgG) (1477 ± 505 pg/mL in control cultures vs. 987 ± 389 pg/mL in cultures with anti-TNF-α antibody; n = 12; P < .01, paired t test). Therefore, TNF-α appeared to up-regulate IL-10 production by M. avium--infected monocytes.

**Effect of IL-10 on induction of other cytokines by M. avium--infected monocytes.** Previous studies have shown that IL-10 inhibits TNF-α and IL-1/β production by LPS-activated human macrophages [16, 17]. The modulation of cytokine production of M. avium--infected monocytes by IL-10 was examined next. Monocytes adherent to plastic were infected with M. avium or stimulated with LPS (1 μg/mL) and cultured for 24 h with or without 1–20 ng/mL IL-10 (final concentrations). IL-10 alone induced minimal cytokine expression (data not shown). Figure 4 shows the concentrations of TNF-α, IL-1/β, and IL-6 in supernatants of monocytes cultured with various concentrations of IL-10 after infection with the pathogenic M. avium LR114 SmT or after stimulation with LPS. Coculturing with IL-10 inhibited TNF-α, IL-1/β, and IL-6 production by monocytes both infected with M. avium and stimulated with LPS in a dose-dependent manner (figure 4). IL-10 itself did not, however, inhibit IL-10 expression (data not shown). The presence of 5 ng/mL IL-10 (final concentration) significantly inhibited TNF-α, IL-1/β, and IL-6 production by M. avium--infected monocytes (75.7% ± 2.7%, 48.1% ± 5.4%, and 57.8% ± 4.8% inhibition, respectively; n = 3; P < .01 vs. cultures without IL-10).

**Effect of IL-10 on intracellular M. avium growth in human monocytes.** We examined the modulation of intracellular M. avium growth by IL-10 in human monocytes. Freshly isolated monocytes were adhered to 96-well tissue culture plates and then

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*Figure 3.* Expression of IL-10--specific mRNA from monocytes infected with 5 M. avium strains. RNA was extracted from lysates of monocytes cultured overnight after infection. Data are mRNA expression by monocytes from 2 independent experiments using 2 subjects. A, Ethidium bromide staining and UV transillumination; B, Southern blot. HPRT, hypoxanthine phosphoribosyl transferase; SmT, smooth, flat, and transparent; SmD, smooth, domed, and opaque.
The intracellular *M. avium* growth in monocytes preexposed to IL-10 before infection was examined next. Monocyte monolayers were pretreated with 10 ng/mL IL-10 for 2 days prior to infection with LR114SmT. After infection, monocytes were cultured for 7 days without IL-10. Pretreatment with IL-10 did not increase intracellular *M. avium* growth in human monocytes (table 3).

We next examined the effects of anti-IL-10 antibody on intracellular *M. avium* growth in human monocytes. Immediately after infection with *M. avium*, a single dose of anti-human IL-10 monoclonal antibody clone JES-3-9D (1–20 µg/mL, final concentration) was added to monocyte cultures. Intracellular *M. avium* growth was assessed by cfu assay. Anti-IL-10 antibody failed to modulate intracellular *M. avium* growth in human monocytes (data not shown).

### Discussion

In this study, we examined IL-10 and TNF-α production by human monocytes infected with *M. avium* and the effect of IL-10 monoclonal antibody clone JES-3-9D (1–20 µg/mL, final concentration) was added to monocyte cultures. Intracellular *M. avium* growth was assessed by cfu assay. Anti-IL-10 antibody failed to modulate intracellular *M. avium* growth in human monocytes (data not shown).

**Table 2.** Effect of recombinant human IL-10 on intracellular *M. avium* growth in human monocytes.

<table>
<thead>
<tr>
<th>IL-10 (ng/mL)</th>
<th>LR114SmT</th>
<th>86m2096</th>
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<tbody>
<tr>
<td>0</td>
<td>4.0 ± 2.0</td>
<td>8.0 ± 2.6</td>
</tr>
<tr>
<td>1</td>
<td>4.3 ± 2.6</td>
<td>4.1 ± 1.7</td>
</tr>
<tr>
<td>10</td>
<td>3.9 ± 2.5</td>
<td>4.1 ± 1.8</td>
</tr>
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NOTE. Data are mean ± SE of colony forming units (cfu) in monocyte lysates of 6 donors for strain LR114SmT (smooth, flat, and transparent) and 4 for strain 86m2096.

**Table 3.** Effect of pretreatment of monocytes with IL-10 on intracellular *M. avium* growth.

<table>
<thead>
<tr>
<th></th>
<th>Mean cfu in monocyte lysates (10³/mL)</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
</tr>
<tr>
<td>IL-10</td>
<td>3</td>
</tr>
</tbody>
</table>

NOTE. Monocytes were preincubated with or without IL-10 for 2 days prior to infection. After infection with strain LR114SmT (smooth, flat, and transparent), monocytes were cultured for up to 7 days with medium alone. Data are mean ± SE.
10 on intracellular *M. avium* growth. Infection with *M. avium* induced IL-10 release from infected human monocytes that peaked 24–48 h after infection. Significant strain-to-strain variation in IL-10 production was seen but did not correlate with the pathogenicity or colonial morphology of the infecting *M. avium* strains. We previously demonstrated that infection with the SmD morphotype of *M. avium* induced more TNF-α expression than did the SmT morphotype and that TNF-α-specific mRNA expression was dissociated from TNF-α protein concentrations in culture supernatants [5]. IL-10–specific mRNA expression paralleled IL-10 concentrations in monocyte supernatants for 4 of 5 *M. avium* strains tested, and there was no relationship with colonial morphotype.

In a murine model, in vivo administrations of multiple doses of anti-IL-10 antibody increased effector function against *M. avium* infection [17, 18]. IL-10 inhibited the effector function of GM-CSF or TNF-α–activated murine macrophages against *M. avium* [17] and increased *Mycobacterium tuberculosis* replication in cultured bone marrow–derived macrophages activated by IFN-γ when IL-10 was added before cytokine activation [17, 23]. Using the in vitro human monocyte infection model, however, exogenous IL-10 added to monocyte cultures immediately after infection with the pathogenic *M. avium* LR114SmT strain and the AIDS-associated isolate 86m2096 and pretreatment of monocytes with IL-10 prior to infection failed to modulate intracellular *M. avium* growth. Furthermore, coculture of infected monocytes with anti-IL-10 antibody did not modulate intracellular *M. avium* growth in vitro. In this human model, therefore, at the concentrations tested, IL-10 may have little role in regulating bactericidal activity of monocytes that have already been infected with *M. avium*.

IL-10, a macrophage-deactivating cytokine, has been shown to inhibit IL-1, IL-6, TNF-α, and GM-CSF production by LPS- or IFN-γ–activated human monocytes [14–16]. Pretreatment of human monocytes with IL-10 (10 μg/mL) inhibited TNF-α and IL-10 production [24]. Malefyt et al. [13] also demonstrated autoregulatory effects of IL-10, specifically, inhibition of IL-10 production by cells incubated with 100 U/mL (200 ng/mL) IL-10. In the experiments reported herein, we demonstrated that coculture with 5 ng/mL IL-10 strongly inhibited IL-10 production by human monocyte-derived macrophages, and the levels produced correlated with mycobacterial virulence [30]. It appears, therefore, that TGF-β may have a more substantial role in regulation of intracellular mycobacterial replication in human mononuclear phagocytes than IL-10 or IL-4.

TNF-α is an important proinflammatory cytokine that mediates granuloma formation, one of the most important host defense mechanisms during infection with intracellular pathogens [31, 32]. TNF-α also has macrophage-activating factor activity against *M. avium* infection [9, 33]. In the current study, and consistent with earlier observations [5], TNF-α production increased when the ratio of infecting organisms was increased and was significantly higher in monocytes infected with SmD morphotype strains, which replicated less in human monocytes than did SmT morphotypes [4].

TNF-α, but not IL-1β or IL-6, also regulates IL-10 production by inducing IL-10 expression and augments LPS-induced IL-10 expression by human monocytes [22]. We also showed that anti-human TNF-α antibody decreased IL-10 release by *M. avium*–infected monocytes. Although TNF-α appeared to up-regulate endogenous IL-10 production, there was no apparent relationship between TNF-α and IL-10 induction by *M. avium* strains (table 1), indicating that factors other than TNF-α also regulate IL-10 production by monocytes infected with *M. avium*.

In our system, exogenous IL-10 and anti-IL-10 antibody failed to modulate intracellular *M. avium* growth in human monocytes. The local balance of cytokines appears to be an important factor in the modulation of intracellular *M. avium* growth in human monocytes; TNF-α and IFN-γ inhibit intracellular *M. avium* replication [4, 8, 9, 33], whereas IL-1α and IL-6 increase intracellular *M. avium* growth [7]. IL-10 inhibited not only the release of macrophage-activating cytokines such as TNF-α but also the expression of intracellular *M. avium* growth-enhancing cytokines (IL-1 and IL-6). Overall, there was no net effect of exogenous IL-10 in inhibiting intracellular *M. avium* growth in this human monocyte infection model. Decreased TNF-α production by monocytes infected with pathogenic SmT morphotype *M. avium* strains, therefore, is potentially more important in *M. avium* pathogenesis than is modulation of IL-10.

References


12. Prete GO, Carli MD, Almerigogna F, Giudizi MG, Biagiotti R, Romagnani S. Human IL-10 is produced by both type 1 helper (Th1) and type 2 helper (Th2) T cell clones and inhibits their antigen-specific proliferation and cytokine production. J Immunol 1993;150:353–60.


