Transgenic Mice Expressing Human Interleukin-10 in the Antigen-Presenting Cell Compartment Show Increased Susceptibility to Infection with *Mycobacterium avium* Associated with Decreased Macrophage Effector Function and Apoptosis

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Interleukin-10 (IL-10) is thought to play an important role in the regulation of microbial immunity. While T-cell-derived IL-10 has been shown to suppress cell-mediated immunity, there has been debate as to whether antigen presenting cell (APC)-derived cytokine can perform the same function in vivo. To assess the influence of APC-produced IL-10 on host resistance to mycobacterial infection, transgenic mice expressing human IL-10 under the control of the major histocompatibility complex class II promoter (hu10Tg) were infected with *Mycobacterium avium*, and bacterial burdens and immune responses were compared with those observed in wild-type (wt) animals. Hu10Tg mice harbored substantially higher numbers of *M. avium* and succumbed 16 to 18 weeks postinfection. The granulomas in infected hu10Tg mice showed marked increases in both acid-fast bacilli and host macrophages. In addition, these animals displayed a dramatic increase in hepatic fibrosis. The increased susceptibility of the hu10Tg mice to *M. avium* infection is independent of T-cell-produced endogenous murine IL-10, since bacterial burdens in mice derived by crossing hu10Tg mice with murine IL-10-deficient mice were not significantly different from those in hu10Tg mice. Importantly, gamma interferon (IFN-γ) responses were not decreased in the infected transgenic animals from those in wt animals, suggesting the normal development of Th1 effector cells. In contrast, mycobacterium-induced macrophage apoptosis as well as production of TNF, nitric oxide, and IL-12p40 were strongly inhibited in hu10Tg mice. Together, these data indicate that APC-derived IL-10 can exert a major inhibitory effect on control of mycobacterial infection by a mechanism involving the suppression of macrophage effector function and apoptosis.

Mycobacteria are intracellular pathogens that primarily invade phagocytes. Activation of infected macrophages and control of mycobacterium replication is essentially dependent on gamma interferon (IFN-γ) produced by T lymphocytes (17). Some bacilli, however, resist killing and survive within macrophages in the face of strong T-cell responses. Mechanisms that alter host immune functions are thought to contribute to the persistence of mycobacterial infection. For example, mycobacteria can inhibit bacterial antigen processing by preventing phagosome maturation (1, 8). In addition, the organisms may evade immune clearance by suppressing macrophage apoptosis (4, 19). Finally, the induction of down-regulatory cytokines, such as interleukin 10 (IL-10) and transforming growth factor β, that can inhibit IFN-γ production by T cells and/or macrophage activation (15, 23, 37, 41), may also contribute to the long-term intracellular survival of the pathogen.

IL-10 is of special interest as a possible evasion strategy because of its strong induction during mycobacterial infection (2, 5, 6, 10, 11). IL-10 profoundly inhibits a broad spectrum of macrophage functions, including monokine synthesis, nitric oxide (NO) production, and expression of costimulatory molecules (31). While IL-10-deficient mice in most studies do not show markedly enhanced resistance to mycobacterial infection, such observations do not rule out a role for IL-10 as one of several redundant mechanisms regulating host resistance to these microorganisms.

Overproduction of IL-10 by T cells has been associated with suppressed immunity and increased susceptibility to mycobacterial infection in both humans and mice (6, 29). In addition to T lymphocytes, antigen-presenting cells (APC) (i.e., dendritic cells, macrophages, and B lymphocytes) are a major source of IL-10 in the immune response to mycobacterial infection (10, 16). IL-10 produced by APC could play a major role as an autocrine regulator of macrophage activation controlling clearance of intracellular bacteria and when triggered during the early stages of host-mycobacterium encounter could strongly influence the generation of effector T cells during the subsequent adaptive response.

In this study we have evaluated the possible influence of APC-derived IL-10 on host resistance to mycobacterial infection by studying the course of *Mycobacterium avium* infection in transgenic mice expressing human IL-10 (hu10Tg) under the
control of the major histocompatibility complex class II (Ea) promoter. These animals constitutively express the cytokine at low levels in macrophages, dendritic cells, and B cells but not in the T-lymphocyte compartment (22). Since human IL-10 is fully functional in mice yet is immunologically distinct from murine IL-10, one can study its effects in vivo and distinguish them from those of the endogenous cytokine. In addition, because the cytokine is constitutively expressed, these animals offer the opportunity to elucidate the effects of IL-10 when it is present at the onset of mycobacterial infection, a situation that may occur in humans coinfected with other pathogens known to stimulate IL-10 production by macrophages (38).

We found that hu10Tg mice show enhanced susceptibility to M. avium infection as well as augmented immunopathology, effects that appear to result primarily from the inhibition of macrophage apoptosis and activation. These findings indicate that APC-derived IL-10 can be an important factor influencing mycobacterial growth as well as the persistence of the pathogen in latently infected hosts.

MATERIALS AND METHODS

Mice. BALB/c, IL-10−/− (on BALB/c background), hu10Tg (22), and hu10Tg/mmu IL-10−/− mice (generated by crossing hu10Tg and IL-10−/− mice) were bred at Tacoma Farms (Germantown, N.Y.). Human IL-10-expressing, heterozygous hu10Tg and hu10Tg/mmu IL-10−/− mice were identified by PCR screening with human IL-10-specific primers (22). Female mice, 8 to 12 weeks old, were used in all experiments.

Bacteria and soluble mycobacterial antigens. M. avium (strain 2-151-SmT) (12) bacilli harvested from infected wild-type (wt) mice were expanded once in Middlebrook 7H9 liquid medium supplemented with albumin-dextrose-catalase (Difco, Detroit, Mich.) for 7 days at 37°C. Aliquots of the bacteria were stored at −70°C. To prepare M. avium antigens (MVAgAg) for in vitro assays, the mycobacteria were lysed by repeated sonication and the soluble fraction was collected following centrifugation (2,000 × g, 15 min) of the bacterial lysates. The resulting preparation was sterile filtered, and the protein concentration was determined by a bicinchoninic acid protein assay (Pierce, Rockford, Ill.) according to the manufacturer’s instructions.

M. avium infection and quantitation of bacterial load. Mice were infected intravenously (i.v.) with 5 × 108 CFU of M. avium. Bacterial loads in infected mice were determined at various time points following infection. Spleens and lungs were homogenized in 2 ml of sterile water, and 100-μl aliquots were used for preparing serial dilution of the bacteria. The diluted homogenates were plated on oleic acid-albumin-dextrose-catalase-supplemented Middlebrook 7H11 Bacto agar (Difco), and colonies were counted visually after 14 days. The bacterial load was expressed as log10 CFU.

Histopathology and quantitation of fibrosis and apoptosis in tissues. Tissue sections from livers, spleens, and lungs were fixed with formalin, sectioned, and stained with hematoxylin and eosin. The Ziehl-Neelsen method was used to stain acid-fast mycobacteria in tissue sections. Collagen deposition in livers, determined as hydroxyproline, was measured as previously described (7). Apoptosis and acid-fast mycobacteria in tissue sections. Collagen deposition in livers, determined as hydroxyproline, was measured as previously described (7). Apoptosis was measured by immunochemical detection using the terminal deoxynucleotidyl transferase–UTP–nick-end-labeling (TUNEL) assay using a commercial kit (Boehringer-Mannheim, Indianapolis, Ind.), and colonies were counted visually after 14 days. The bacterial load was expressed as log10 CFU.

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RESULTS

M. avium-infected hu10Tg mice develop abnormal granulomas and increased tissue fibrosis. Since the granulomatous response is thought to play a critical role in the control of mycobacterial dissemination, we next examined if granuloma formation and the extent of bacterial replication within these tissue lesions differ between infected wt and transgenic mice. While changes

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were evident in both pulmonary and splenic granulomas, the lesions in liver presented the most obvious differences because of their compact morphology. In that tissue at 8 weeks of infection, granulomas were significantly \((P = 0.04)\) larger in hu10Tg mice (mean diameter \(\pm\) standard deviation \([\text{SD}]\) = 102.4 \(\pm\) 19.3 \(\mu\)m) than those in wt animals (71.2 \(\pm\) 15.6 \(\mu\)m). Moreover, the granulomas in hu10Tg mice typically contained more macrophages (65.8\% \(\pm\) 4.4\%) than the wt animals (55.5\% \(\pm\) 6.7\%; \(P = 0.04\)), with many of those in the transgenic mice displaying a foamy cytoplasm (Fig. 2A and B). A corresponding difference in the level of lymphocytes in the granulomas of the hu10Tg and wt mice was also observed (32.5\% \(\pm\) 2.9\% versus 42.0\% \(\pm\) 5.7\%; \(P = 0.03\)). In addition, multinucleated giant cells were frequently observed in the granulomas of transgenic but not wt animals. Strikingly, the granulomas in hu10Tg mice contained large numbers of bacilli compared to those in wt animals, in which only a few bacilli were detected by acid-fast staining (Fig. 2C and D). The number of mycobacteria per cell also increased in the granulomas of hu10Tg mice, in keeping with the elevated numbers of CFU observed in these animals, suggesting a loss of control of mycobacterial replication within infected macrophages.

As an additional parameter of the tissue response, we quantified fibrosis (as measured by hydroxyproline levels) in the livers of the two groups of animals. Hepatic fibrosis was found to be dramatically increased in the livers of the infected hu10Tg mice (Fig. 3).

**Increased susceptibility of hu10Tg mice to *M. avium* infection is not the result of dysregulated endogenous murine IL-10 expression.** Since hu10Tg mice also express muIL-10, it was possible that the enhanced susceptibility of these animals to *M. avium* infection was due to abnormal expression of the endogenous murine cytokine. For this reason we compared *M. avium*-induced muIL-10 production in wt and hu10Tg mice by restimulating splenocytes with MAVAg in vitro and measuring cytokine levels in culture supernatants. As previously reported (2, 11), muIL-10 production by spleen cells steadily increased during the first 3 months of *M. avium* infection, reaching similar levels in the hu10Tg and wt animals (Fig. 4A). Indeed, if anything, production of muIL-10 was delayed in the hu10Tg mice. Results of T-cell enrichment experiments argued that T lymphocytes are a major cellular source of muIL-10 in the splenic cultures of infected animals, and T-cell depletion using anti-Thy1.2 magnetic-activated cell separation beads resulted in a greater than 95% reduction in the level of murine IL-10 produced by MAVAg-stimulated splenocytes (data not shown).

To formally rule out the contribution of muIL-10 to the increased susceptibility of hu10Tg mice, we compared bacterial burdens in these animals with those in hu10Tg/muIL-10 \(/^{--}\) mice derived by crossing hu10Tg with IL-10 knockout mice. As shown in Fig. 5A, infected hu10Tg/muIL-10 \(/^{--}\) mice developed splenic bacterial burdens indistinguishable from those found in hu10Tg animals in which endogenous muIL-10 expression is intact. Moreover, in vivo treatment of infected hu10Tg/muIL-10 \(/^{--}\) mice with neutralizing MAb specific for human IL-10 MAb the day before infection and on a weekly basis thereafter resulted in a reduction in bacterial loads (Fig. 5B).

**Infected hu10Tg mice generate normal mycobacterium-specific Th1 responses.** Since IFN-\(\gamma\) produced by Th1 cells is essential for the activation of macrophages to control mycobacterial replication, we examined if the increased susceptibility of hu10Tg mice to *M. avium* infection is the result of effects of huIL-10 on the generation of Th1 responses. We first examined IFN-\(\gamma\) production by unfractionated splenocytes restimulated in vitro with MAVAg. As shown by previous studies (2, 11), IFN-\(\gamma\) levels rapidly increased, reaching peak levels at 2 weeks p.i., and then returned to near baseline levels. The same kinetics of IFN-\(\gamma\) production was observed in wt and hu10Tg mice, the latter animals producing more IFN-\(\gamma\) at 2 weeks than the former. This suggests that suppression of IFN-\(\gamma\) production is not the primary cause of the reduced control of *M. avium* infection observed (Fig. 5A). To directly compare intrinsic T-lymphocyte responses in the two groups of animals, enriched splenic T cells were restimulated with MAVAg in the presence of APC from uninfected mice. Interestingly, while production of the cytokine was suppressed in cultures of unfractionated spleen cells at 8 weeks p.i., enriched T cells from the same spleens produced high levels of IFN-\(\gamma\) when provided with APC from naïve animals, and this response was comparable in the wt and transgenic animals (Fig. 5B). The latter
observations suggest that the suppressed IFN-γ production observed during late infection is the result of altered APC function. Attempts to reverse this suppression by addition of anti-IL-10R MAb were unsuccessful (data not shown), indicating that IL-10 production by APC is not in itself the explanation for the observed down-regulation of IFN-γ production. To further confirm the normal activation of effector CD4+ T lymphocytes in the infected hu10Tg mice, we quantitated the numbers of activated (CD44hi) CD4+ T cells in spleens. No significant differences were observed between the wt and transgenic animals at either week 2 (6.2 × 10^6 ± 0.2 × 10^6 versus 5.9 × 10^6 ± 0.3 × 10^6; n = 4) or week 8 (6.8 × 10^6 ± 0.4 × 10^6 versus 7.1 × 10^6 ± 0.5 × 10^6; n = 4) p.i.

Splenic macrophages from infected hu10Tg mice fail to undergo apoptosis and produce lower levels of proinflammatory mediators. Since Th1 development and lymphokine production appear to be normal in hu10Tg mice, we next asked whether defects in macrophage survival and/or function could explain the increased susceptibility to M. avium infection. To compare macrophage numbers, splenocytes from wt and transgenic animals were stained with anti-Gr-1 and CD11b MAb ex vivo and analyzed by flow cytometry. Although initially similar, by 8 weeks p.i., spleens of transgenic mice contained approximately twice the percentage of Gr-1−CD11b+ cells as their wt counterparts (Fig. 6A). To investigate whether this increase is due to a difference in macrophage survival in M. avium-infected hu10Tg mice, we measured apoptosis of splenic Gr-1−CD11b+ cells in infected wt and transgenic animals by flow-cytometric staining utilizing Annexin V-FITC. The percentage of apoptotic splenic macrophages in infected wt mice had increased more than twofold by 8 weeks p.i., while no increase was observed in the same populations from infected hu10Tg animals (Fig. 6B and C). Examination of tissue sections using TUNEL staining confirmed that, in contrast to the situation in wt mice, there was no significant difference in the number of TUNEL-positive cells in the spleens of transgenic mice before and 8 weeks after M. avium infection (data not shown).

We next assessed the production of the proinflammatory mediators TNF, NO, and IL-12 as markers of macrophage activation. MAVAg stimulated spleen cells from hu10Tg mice

FIG. 2. Hepatic granulomas in M. avium-infected hu10Tg mice contain increased numbers of multinucleated giant cells and abundant mycobacteria. Eight weeks after infection with M. avium, liver tissues from wt (A and C) or hu10Tg (B and D) mice were collected and fixed with formalin. Paraffin-embedded sections were stained with hematoxylin and eosin (A and B; magnification, ×200). The Ziehl-Neelsen method was used to stain acid-fast bacilli (C and D; magnification, ×200). The insert in panel B shows two multinucleated giant cells in hepatic granulomas of hu10Tg mice (magnification, ×630).
produced significantly less TNF, NO, and IL-12p40 than those from wt animals, particularly at the early stages of infection (data not shown). To confirm that these differences are not the result of impaired T-cell function, we analyzed the production of the same mediators by a macrophage-enriched, T-cell-depleted, adherent subpopulation of spleens from mice at 2 weeks p.i. When derived from hu10Tg mice, these cells produced lower levels of TNF, NO, and IL-12p40 in vitro than the equivalent macrophage-enriched fraction from wt animals (Fig. 6D). These observations suggest that the decreased resistance of hu10Tg mice to \textit{M. avium} infection is the result of IL-10-mediated deactivation of macrophages combined with increased survival of these cells, a conclusion consistent with the histopathologic observation of increased numbers of macrophages as well as bacilli per macrophage within granulomas in these animals (Fig. 2D).

\section*{DISCUSSION}

IL-10 is a prominent cytokine in the immune responses of both humans and mice to mycobacteria (38). Both T cells and APC produce IL-10 in response to mycobacterial infection (10, 21, 29, 32, 41). Interestingly, although originally described as a Th2 cytokine, IL-10 appears to be produced in large amounts during mycobacterial infection by Th1-type interferon-producing CD4\(^+\) lymphocytes (20, 28). That T-cell-derived IL-10 can play a role in regulating host resistance to mycobacterial infection was confirmed in a study demonstrating enhanced susceptibility to \textit{Mycobacterium bovis} BCG infection of transgenic mice overproducing IL-10 in the T-lymphocyte compartment (34). While the induction of T cells producing IL-10 can be readily measured by in vitro recall with mycobacterial antigen, secretion of the cytokine by pathogen-stimulated APC can be assayed only as constitutive production, which is often difficult to detect in vitro. An important question concerns whether IL-10 produced by APC can also influence the outcome of mycobacterial infection. In the present study, we used transgenic mice expressing huIL-10 under the control of the major histocompatibility complex class II promoter to address this issue. Because human IL-10 is fully functional in mice, the relative contributions of APC and T-cell-derived IL-10 can be distinguished in vivo.

\textit{M. avium}-infected hu10Tg mice developed higher bacterial burdens than control animals and succumbed precipitously at 16 to 18 weeks of infection (Fig. 1), an outcome unusual for mice exposed to this normally nonlethal bacterial strain. In addition, hu10Tg/muIL-10\(^{-/-}\) mice were determined (B). Bacterial burdens in hu10Tg/muIL-10\(^{-/-}\) mice were compared. The mean (± SD) numbers of CFU in spleens of 4 to 5 individual mice are shown. The Student \(t\) test was used to analyze the statistical difference between group means.

\begin{figure}[h]
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\caption{Hu10Tg mice develop severe hepatic fibrosis following \textit{M. avium} infection. Hepatic fibrosis, measured as hydroxyproline, was compared for the wt and hu10Tg mice. The means (± SD) for four animals are shown. The significance of differences between wt and hu10Tg mice was determined using the Student \(t\) test (*, \(P < 0.05\)). Data are representative results from one of two similar experiments.}
\end{figure}

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure4.png}
\caption{Increased susceptibility of hu10Tg mice to \textit{M. avium} infection is independent of endogenous production of murine IL-10. Splenocytes of wt and hu10Tg mice isolated at weeks 0, 2, 4, 8, and 12 p.i. were restimulated in vitro with MAVAg. The mean (± SD) muIL-10 secreted in duplicate cultures is shown (A). Eight weeks after i.v. infection with \(5 \times 10^6\) CFU of \textit{M. avium}, bacterial loads in the spleens of wt, hu10Tg, muIL-10\(^{-/-}\), and hu10Tg/muIL-10\(^{-/-}\) mice were determined (B). Bacterial burdens in hu10Tg/muIL-10\(^{-/-}\) mice treated with control (GL113) or anti-huIL-10 (JES3-19F) MAb were also compared. The mean (± SD) numbers of CFU in spleens of 4 to 5 individual mice are shown. The Student \(t\) test was used to analyze the statistical difference between group means.}
\end{figure}
bacterial loads and ensuing tissue damage occurring in these animals.

IL-10 is known to have an indirect suppressive effect on the development of Th1 cells and on their expression of IFN-γ (9, 14). That this mechanism might be operating in mycobacterial infection is suggested by our observations (Fig. 4 and 5) and those of others (2, 11) showing that T-cell-derived IFN-γ production in mice infected with M. avium decreases as IL-10 levels increase. Nevertheless, a number of other findings argue that suppression by IL-10 is not the explanation of this decline in IFN-γ production. For example, IL-10−/− mice infected with either BCG (35) or M. avium (D. Jankovic, unpublished data) do not show markedly increased levels of IFN-γ during chronic infection. The results reported here demonstrate that the increased APC-derived IL-10 in our hu10Tg mice fails to suppress the early T-cell-derived IFN-γ response induced by M. avium infection (Fig. 5). Taken together, these observations indicate that the down-regulation of IFN-γ production seen in M. avium infection must be the result of immunoregulatory mechanisms distinct from IL-10 production but dependent on APC, since replacement of APC from infected mice with those from naive animals restores in vitro IFN-γ production to high levels (Fig. 5B). These findings also argue that any effects of IL-10 on host resistance must operate at a stage of the antymycobacterial effector mechanism distinct from the T-cell response.

Histological and flow-cytometric examination of infected livers and spleens revealed several important clues concerning the mechanisms underlying the increased susceptibility of hu10Tg mice to M. avium infection. First, macrophage recruitment into granulomas in the transgenic animals is clearly enhanced, with many of these cells displaying extended, foamy cytoplasm. This elevation in macrophage numbers was associated with a block in apoptosis (Fig. 6). Recent findings demonstrating that virulent mycobacterial strains induce significantly less macrophage apoptosis than the attenuated strains suggest that the induction of apoptosis in infected macrophages is an important innate defense mechanism against mycobacterial infections (18, 26, 30). Apoptosis prevents the release of intracellular components and the spread of the infection by sequestering the pathogens within apoptotic bodies. It has been previously established that IL-10 produced by

FIG. 6. Splenic macrophages from M. avium-infected hu10Tg mice fail to undergo apoptosis and produce less TNF, NO, and IL-12p40 than their wt counterparts. Percentages of Gr-1⁺CD11b⁺ macrophages in spleens (n = 4) of wt (open bars) and hu10Tg (closed bars) mice were compared ex vivo by flow cytometry before infection and 8 weeks p.i. (A). Splenocytes from the same animals were also costained with Annexin V FITC and PI, as well as MAb to Gr-1 and CD11b to determine the percentage of apoptotic macrophages (B and C). Representative FACS profiles showing costaining of Annexin V-FITC and PI on splenic Gr-1⁺CD11b⁺ macrophages from mice at 8 weeks p.i. (B, top panels) are presented. The numbers indicate the percentages of Annexin V-FITC⁺PI⁻Gr-1⁺CD11b⁺ cells. Preincubation of the same cells with purified recombinant Annexin V blocked Annexin V-FITC binding, confirming the specificity of the staining (B, lower panels). The mean (± SD) percentages of apoptotic macrophages (n = 4) in spleens of wt and hu10Tg mice before infection and 8 wk p.i. are shown in Fig. 6C. To measure macrophage production of proinflammatory mediators (D), T-cell-depleted, splenic adherent cells isolated from wt (open) or hu10Tg (closed) mice 2 wk after infection with M. avium were restimulated with MAVAg in vitro for 72 h. Secreted TNF and IL-12 was measured by ELISA, and NO was measured by Greiss reagent. The data shown are the means (± SD) for triplicate cultures performed on pooled cells from three animals. The significance of differences between wt and hu10Tg mice was determined using the Student t test (*, P < 0.05).
mycobacterium-infected macrophages inhibits apoptosis of these cells by suppressing TNF function (3, 4). Consistent with these in vitro studies, the attenuated macrophage apoptosis in infected hu10Tg mice is associated with overproduction of IL-10 by APC and reduced secretion of TNF by splenic macrophages (Fig. 6). APC-derived IL-10 therefore protects M. avium from immune clearance by providing a supportive environment for its rapid growth and spread.

A second important histological finding is the appearance of greatly increased numbers of bacilli at both the single-macrophage and whole-granuloma levels in the tissues of transgenic mice (Fig. 2), suggesting a failure to restrict bacterial growth rather than defective containment of the organism within these lesions (Fig. 2). This finding is consistent with the direct suppression of macrophage control of mycobacterial growth by APC-derived IL-10, a hypothesis supported by our observation of reduced production of TNF, NO, and IL-12p40 by macrophages from infected IL-10-transgenic mice. These inflammatory mediators, while not directly implicated in the mechanism of M. avium (as opposed to BCG and M. tuberculosis) growth restriction, are nevertheless useful markers of macrophage activation.

Since IL-10 has a major down-regulatory effect on cell-mediated immunity, it has been hypothesized that the production of this cytokine helps promote the long-term survival of mycobacteria in chronically infected hosts (33, 38, 40). Studies directed at defining the function of IL-10 in mycobacterial function have in general supported this concept but failed to establish a major function for IL-10 production in the prevention of bacterial clearance. Thus, mice deficient in IL-10 show increased resistance to M. avium (39) and in some (25, 35, 39) but not all (13, 36) studies display enhanced early control of M. tuberculosis and BCG infection. Although IL-10−/− mice have never been shown to exhibit dramatically reduced bacterial burdens, our observations and those of others (27, 34) that excess IL-10 production can promote intracellular pathogen growth in macrophages argue that IL-10-mediated immune down-regulation may contribute to the maintenance of latency in chronic mycobacterial infection, possibly as one of several redundant mechanisms.

Our conclusion that infected hu10Tg mice display impaired macrophage effector function while developing normal Th1 responses is in agreement with results of two recent studies employing transgenic mice in which IL-10 was overexpressed under the control of either the CD2 enhancer (34) or the macrophage cell surface molecule CD68 (27). These animals displayed IL-10-mediated deactivation of macrophage function but unimpaired IFN-γ responses and exhibited decreased resistance to BCG infection. Together, the present and previous studies support the argument that the immunosuppressive effects of IL-10 occur primarily at the level of effector cell activity rather than lymphokine-producing T cells (31). This conclusion has important implications for the measurement of protective antimycobacterial responses. Thus, as clearly demonstrated here and in the previous studies of mycobacterial infection in IL-10 transgenic mice, the assay of T-cell IFN-γ production may not accurately reflect host immune status when macrophage down-regulatory cytokines are simultaneously induced. This concept needs to be considered when using T-cell function as a read-out in vaccination studies.

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