

Blocking the Receptor for IL-10 Improves Antimycobacterial Chemotherapy and Vaccination¹

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Novel approaches are required for the prevention and therapy of mycobacterial infections since the only vaccine in use, bacillus Calmette-Guérin, is poorly effective and chemotherapy is long and often ineffective in sterilizing the infection. We used a mouse model of *Mycobacterium avium* infection to address the usefulness of a mAb able to block IL-10R both in treatment of primary infections and in conventional multidrug therapy and subunit vaccination. Treatment of infected mice with this mAb during the entire period of experimental infection had little impact on the course of *M. avium* infection, with a slight improvement in the resistance of infected mice observed in the liver and spleen at day 30 of infection, which was associated with increased macrophage activation and priming of CD4⁺ T cells for IFN- γ production. Administration of this mAb later in infection had no effect on its course, but improved the effectiveness of chemotherapy when the latter was started in a chronic phase of infection. Also, the anti-IL-10R mAb acted as an adjuvant in the induction of protective immunity upon vaccination with a mycobacterial subunit preparation. *The Journal of Immunology*, 2001, 167: 1535–1541.

Clinical management of mycobacterial infections has always presented difficulties due to the refractoriness of mycobacteria to chemotherapy and to the low efficacy of the only vaccine in use, the attenuated strain of *Mycobacterium bovis* bacillus Calmette-Guérin (BCG).³ Antimycobacterial therapy in tuberculosis is long, taking several months to be completed with the classical regimens (1). Such length in the treatment promotes low compliance with premature interruption of chemotherapy and favoring the emergence of antibiotic resistance. Indeed, the emergence of multidrug resistant strains of *Mycobacterium tuberculosis* has worsened the problem of tuberculosis (2).

Chemotherapy of infections by nontuberculous mycobacteria such as *Mycobacterium avium* complex (MAC) is also difficult due to the low susceptibility of this opportunistic pathogen to most commonly used antitubercle compounds (3). Therapy resorts to a combination of several drugs and, as in tuberculosis, is very lengthy. Long-term treatment with macrolides such as clarithromycin, which is highly effective in the treatment of MAC infections (4, 5), leads to the emergence of resistant forms (6–8), which can be prevented by combining at least two or three drugs (9, 10). However, this in turn can lead to severe side effects (11, 12). Ways to shorten therapeutical courses as well as increasing efficacy of the treatment are thus needed.

Prevention of mycobacterial infections by vaccination is still an area deserving the attention of many due to the low efficacy of

BCG. Analysis of BCG vaccination studies has shown a wide variability in the efficacy of this vaccine against tuberculosis, ranging from 0 to 80% according to geographical area of the study, being often useless in developing countries, in which its success would be of major benefit to the populations (13). One of the strategies currently being studied to improve vaccine efficacy is the use of subunit protein preparations. We have previously used the *M. avium* model to screen for possible immunomodulatory interventions that may increase the protection afforded by the vaccines (14, 15), and found that this model is suitable for such screening similarly to tuberculosis models (16).

IL-10 is a cytokine with pleiotropic activities, being its major effects associated with its antiinflammatory and immunosuppressive properties on hemopoietic cells (17–19). The increased production of this cytokine has been shown to underlie susceptibility to numerous infections (20–23). Among mycobacterial infections, IL-10 was shown to promote susceptibility to BCG and MAC (24–27). The possible mechanisms underlying increased susceptibility to infection may be many different ones, such as modulation of the type and polarization of the immune response; interference with Ag presentation, namely at the level of the dendritic cells; regulation of the immune response; and modulation of the activities on the effector cells, namely deactivation of monocytes/macrophages (17–19). Regarding the latter effects, IL-10 reduces antimicrobial functions of activated macrophages by decreasing the generation of superoxide anion and NO (28, 29). Inhibition of inflammation relates to the inhibition of the production of proinflammatory cytokines and monokines by activated monocytes/macrophages (30, 31) and to the effects on the immune response due to the down-regulation of the expression of costimulatory molecules and of class II molecules (32–35). IL-10 may thus also affect the outcome of vaccination namely to subunit vaccines. Recently, a role for IL-10 in the induction of tolerance to soluble Ag was postulated (36). Thus, we reevaluated the participation of IL-10 in the chemotherapy and vaccination of mycobacteriosis using an established mouse model of infection by *M. avium*. We show that although not explaining susceptibility to infection, IL-10 may be responsible to refractoriness to chemotherapy. Additionally, we postulate a role for inhibitors of IL-10 action as potential adjuvants in subunit vaccines.

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³ Abbreviations used in this paper: BCG, bacillus Calmette-Guérin; CFP, culture filtrate proteins of *M. avium* 2447; DDA, dimethyl dioctadecyl ammonium chloride; MAC, *M. avium* complex; PEC, peritoneal exudate cell.

Materials and Methods

Reagents and Abs

Mycobacterial growth media were purchased from Difco (Sparks, MD). DMEM, RPMI 1640 medium, HEPES buffer, FCS sodium pyruvate, penicillin/streptomycin, L-glutamine, and protein G columns were purchased from Life Technologies (Paisley, U.K.). Tween 80, oleic acid, 2-ME, potassium bicarbonate, BSA, Con A, IFA, and ethambutol were purchased from Sigma (St. Louis, MO). Clarithromycin was purchased from Abbot Laboratories (Amadora, Portugal), and rifabutin (Pharmacia Adria, Dublin, OH) was supplied by the manufacturers. Mouse rIFN- γ was purchased from Genzyme (Cambridge, MA). The IL-10R-blocking IgG1 was obtained from the hybridoma cell line 1B1.2, a kind gift from K. Moore (DNAX, Palo Alto, CA) (37). The anti- β -galactosidase IgG1, an isotype-matched Ig with irrelevant specificity, was obtained from the hybridoma cell line GL113 (DNAX). The IFN- γ -specific IgG1 mAbs were obtained from the hybridomas AN18 (DNAX) and R4-6A2 (American Type Culture Collection, Manassas, VA). Hybridomas were grown in ascites in Harlan Sprague Dawley nude mice primed with IFA. Nonimmune rat IgG were obtained from serum of Lewis rats. Abs were purified by affinity chromatography using a protein G-agarose column, followed by dialysis against PBS. FITC-conjugated rat anti-mouse CD4, PE-conjugated rat anti-mouse CD8, and PE-conjugated rat anti-mouse CD3 were purchased from Pharmingen (San Diego, CA).

Animals and infections

Specific pathogen-free female BALB/c mice were purchased from Harlan Iberica (Barcelona, Spain). Lewis rats and outbred Harlan Sprague Dawley nude mice were purchased from Gulbenkian Institute for Science (Oeiras, Portugal). These mice were kept in sterile housing conditions in cages provided with high efficiency particulate air filter-bearing caps. All mice were used at 6–8 wk of age.

M. avium 2447 SmT (an AIDS isolate obtained from F. Portaels, Institute of Tropical Medicine, Antwerp, Belgium) stored as frozen aliquots at -70°C as well as the inoculum used in experimental infections were prepared as described elsewhere (14). BALB/c mice were infected i.v. with 10^6 CFU of *M. avium* strain 2447 SmT through a lateral tail vein. At different time points, mice were sacrificed by cervical dislocation, and the organs (spleen, liver, and lung) were collected, homogenized, serially diluted in a 0.04% Tween 80 solution in distilled water, and plated onto Middlebrook 7H10 agar medium. The plates were incubated for 1–2 wk at 37°C , and the number of CFU was counted. The data are expressed as the mean of the $\log_{10}$ number of CFU recovered per organ $\pm$ 1 SD ($n = 4$ or 5 animals). Statistical significance of the differences was analyzed by performing the Student's *t* test using unpaired data.

Treatment of mice with mAbs

To block the IL-10R, mice were injected i.p. with 1 mg anti-IL-10R mAb 12 h before infection or 30 days after infection, followed by i.p. injections with 0.2 mg anti-IL-10R mAb every other day during the whole course of infection. Control mice were submitted to the same protocol of administration of Abs, being injected with anti- β -galactosidase mAb (GL113 mAb) as an Ig isotype control or with nonimmune rat IgG.

Antibiotic regimens

Drug therapy was done orally by adding clarithromycin (200 mg/kg/day), rifabutin (40 mg/kg/day), and ethambutol (25 mg/kg/day) in the drinking water. Antibiotic treatment was done in one-half of the animals at the beginning of infection, and the remaining mice received the same therapy regimen 30 days after infection. The treatment was done until the end of the experimental period of infection. Control mice drank nonsupplemented water. The treatment of mice with mAbs was done in the same period of time as the antibiotic chemotherapy.

Ag preparations

Preparation of *M. avium* envelope proteins was performed as described elsewhere (38). Briefly, *M. avium* 2447 was grown for 2 wk in Sauton medium supplemented with 0.5% pyruvate and 0.5% glucose. The pellet obtained by centrifugation was resuspended in PBS containing 0.1% Tween 80, 1 mM MgCl_2 (Merck, Darmstadt, Germany), and 1 mM benzamidine (Sigma), and sonicated with pulses of 1 min at maximum power, keeping the sample in ice during the procedure. The sonicate was centrifuged to discard intact mycobacteria (30 min at $2700 \times g$), and the supernatant was dialyzed against PBS. The latter was then ultracentrifuged for 2 h at $150,000 \times g$, and the pellet, containing the envelope proteins, was resuspended in PBS.

Purification and culture of CD4^+ T cells

Spleens from mice noninfected or that had been infected for 20 days with *M. avium* 2447 treated either with anti-IL-10R mAb or nonimmune rat IgG were aseptically collected. Half of the organ was used to prepare single cell suspensions. Spleen cells were depleted of RBCs by incubation in hemolytic buffer (155 mM NH_4Cl , 10 mM KHCO_3 , pH 7.2) for 5–10 min at room temperature and thoroughly washed. Nucleated spleen cells were pooled ($n = 4$ animals) and incubated (5×10^6 cells/ml) in tissue culture petri dishes at 37°C in a 7% CO_2 atmosphere for 3 h to remove adherent cells. Nonadherent cells were centrifuged; resuspended in PBS containing 0.1% glucose, 1% BSA, and 5 mM EDTA, pH 7.2, at a concentration of 1×10^8 cells/ml; and incubated with microbeads coated with anti-CD4 mAbs (clone L3T4; Miltenyi Biotec, Bergisch Gladbach, Germany) at 4°C for 20 min. Cells were washed and resuspended in PBS-glucose-BSA-EDTA buffer (10^8 cells/500 μl), and CD4^+ T spleen cells were positively selected by using MiniMACS separation columns (Miltenyi), as described in the instructions from the manufacturer. CD4^+ T cells (94–95.2% pure on FACS analysis) were washed, counted, and resuspended in RPMI 1640 containing 10% heat-inactivated FCS, HEPES (10 mM), penicillin (100 U/ml), streptomycin (100 $\mu\text{g}/\text{ml}$), L-glutamine (1%), and 2-ME (0.05 mM). CD4^+ T cells were cultured in triplicate at a density of 1×10^5 cells/well in duplicate 96-well plates with irradiated (3000 rad) nucleated spleen cells as APC (1×10^7 cells/ml) either with no further stimulus or in the presence of Con A (5 $\mu\text{g}/\text{ml}$) or *M. avium* envelope proteins (4 $\mu\text{g}/\text{ml}$) at 37°C in 7% CO_2 atmosphere. Supernatants were collected from triplicate cultures after 72 h and frozen at -20°C until the measurement of IFN- γ by ELISA. The duplicate plates were used in the proliferation assay.

Analysis of the state of activation of peritoneal exudate cells (PEC)

PEC were obtained by washing with cold PBS the peritoneal cavity of noninfected mice or of mice that had been infected for 20 days with *M. avium* 2447 and treated with either anti-IL-10R mAb or nonimmune rat IgG as a control. Cells were washed and resuspended in DMEM containing 10 mM HEPES buffer, 1 mM sodium pyruvate, 1% L-glutamine, and 10% of heat-inactivated FCS, and total cell counts were done. PEC were plated in triplicate at a density of $4\text{--}5 \times 10^5$ cells/well in 96-well flat-bottom plates. After 3 h of incubation (37°C , 7% CO_2), the nonadherent cells were removed by washing three times with prewarmed DMEM. Adherent cells were stimulated for 72 h with LPS (2 $\mu\text{g}/\text{ml}$) at 37°C in a 7% CO_2 atmosphere to study their ability to produce nitrite. The amount of nitrite secreted by the adherent cells and present in supernatants was measured with the Griess reagent, as described elsewhere (39). Briefly, 100 μl Griess reagent (1% sulfanilamide, 0.1% naphthylethylenediamine, and 2.5% H_3PO_4 in distilled H_2O) was added to 100 μl cell-free supernatant. After 10 min of incubation at room temperature, the OD550 was read with an ELISA reader, and the nitrite concentration was determined by the use of a standard curve of NaNO_2 concentrations.

Cell monolayers were lysed by three cycles of freezing and thawing, and the amount of protein in each well was determined by the Micro BCA Protein Assay reagent kit (Pierce, Rockford, IL), according to the instructions of the manufacturer. The data are expressed as the mean of the amount nitrite present in supernatant per mg of adherent cell protein found in each well $\pm$ 1 SD of triplicates from four individual mice for each group.

Flow cytometry

To analyze the CD4^+ T cell-enriched spleen cells from uninfected and infected mice used in culture experiments, 10^6 cells from the purified CD4^+ T cell pools were incubated for 30 min at 4°C in a microtiter plate with PE-conjugated rat anti-mouse CD8 or PE-conjugated rat anti-mouse CD3 and FITC-conjugated rat anti-mouse CD4 mAbs. Cells were washed twice with staining medium (PBS containing 0.1% sodium azide and 3% FCS) and resuspended in staining medium containing propidium iodide to allow exclusion of dead cells. Flow cytometric analysis was performed with a FACScan apparatus (Becton Dickinson, Mountain View, CA) equipped with PCLYSISII software. For each sample, 10,000 events were analyzed.

Cytokine assays

Blood from sacrificed mice was allowed to clot, and serum was collected after centrifugation and frozen at -70°C until use. The spleen and lung homogenates were mixed with 0.1% of Triton X-100 (1:1) and incubated for 2 h at 4°C . After centrifugation, supernatants were collected and frozen at -70°C until use. Quantification of IFN- γ in sera, culture supernatants, and homogenates of spleens and lungs was done by a two-site sandwich ELISA with the anti-IFN- γ -specific affinity-purified mAbs (R4-6A2 as

capture and biotinylated AN-18 as detecting Abs). The sensitivity of the assay was 80 pg/ml.

Proliferation assay

Cell proliferation was measured by [^3H]TdR incorporation. Briefly, proliferative responses were assessed after 48 h of culture in a humidified atmosphere of 7% CO_2 in air. Cultures were pulsed 18–20 h before harvesting with 0.5 μCi [^3H]TdR (Amersham, Little Chalfont, U.K.) per well, and incorporation of [^3H]TdR was measured by liquid scintillation. Results were calculated as mean cpm of triplicate cultures $\pm$ 1 SD ($n = 4$ animals).

Immunization studies

Culture filtrate proteins of *M. avium* 2447 (CFP) were obtained from supernatants of *M. avium* 2447 cultures, as described elsewhere (38). To immunize animals, groups of eight mice were injected s.c. with 45 μg CFP three times at the base of the tail in separate locations, at weekly intervals. CFP was administered either as a solution or emulsified with 250 μg dimethyl diocetadecyl ammonium chloride (DDA; Eastman Kodak, Rochester, NY) as an adjuvant. The control mice were treated with DDA or PBS. Half of the animals in each group received 1 mg anti-IL-10R mAb i.p. 2 h before each immunization, whereas the other half was treated with non-immune rat IgG as a control. Two months after the last immunization, mice were challenged with 10^6 CFU of *M. avium* 2447. The resistance provided by the experimental vaccination was calculated by quantifying the bacterial load in the spleen and the liver homogenates 30 days later, as described above. Values are reported as $\log_{10}$ protection calculated by subtracting the mean of $\log_{10}$ CFU in the immunized mice from the mean of $\log_{10}$ CFU in untreated mice. Statistical significance of the differences was analyzed by performing ANOVA test.

Statistical analysis

Statistical analysis was performed using Student's *t* test or the ANOVA test (*, $p < 0.05$; **, $p < 0.01$).

Results

IL-10 has a minor effect in determining susceptibility to MAC in the mouse model

To assess the effect of blocking the IL-10R during *M. avium* infections, BALB/c mice were infected with *M. avium* 2447 and treated throughout the infection with either anti-IL-10R mAb or nonimmune rat IgG, as described in *Materials and Methods*. The growth of *M. avium* was monitored for 90 days in the spleen, liver, and lung of infected animals. As shown in Fig. 1A, the treatment with the blocking Ab had a small impact on the course of the infection, leading to an increased resistance 1 mo after infection in the liver (0.79 $\log_{10}$ less mycobacteria) and spleen (0.55 $\log_{10}$ less mycobacteria), which disappeared later in the chronic phase of the infection.

Given that IFN- γ is essential in the resistance against infection by *M. avium* 2447 (40, 41) and that IL-10 has been described as an antiinflammatory cytokine with an ability to indirectly inhibit IFN- γ production (17–19), we evaluated the levels of the latter cytokine in the sera and in homogenates of spleens and lungs from infected mice. As observed in Fig. 1B, treatment with the anti-IL-10R mAb increased the levels of IFN- γ at different time points of infection as compared with control mice. This was particularly evident in serum at day 30 of infection and in spleen at day 60 of infection, in which differences in the amount of IFN- γ between both groups were more than 2-fold.

To analyze the importance of IL-10 in the late phase of infection, mice were infected with *M. avium* and treated with either anti-IL-10R mAb or control IgG starting on day 30 of infection. In this experiment, no differences in the bacterial loads were found between the two groups of animals at 90 days of infection either in liver, spleen, or lung (Fig. 1C).

Effect of anti-IL-10R mAb treatment on the immune response

The previous data suggested that the major effect of anti-IL-10R mAb treatment occurs early, i.e., in the first month of infection.

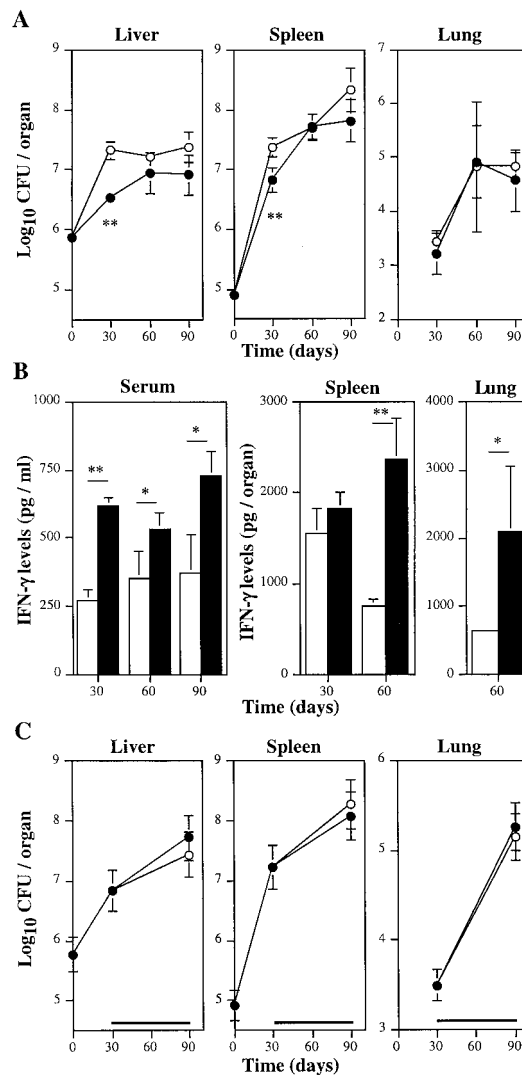


FIGURE 1. Administration of the anti-IL-10R mAb has a minor effect on early resistance to *M. avium* infection. Animals were infected i.v. with 10^6 CFU of *M. avium* 2447 and treated with anti-IL-10R mAb (● and ■) or nonimmune rat IgG as a control (○ and □). Treatment of mice was started at the beginning (A and B) or at day 30 (C) of infection, and was done until the end of experimental *M. avium* infection. A and C, Enumeration of viable bacteria recovered from the organs of infected mice at different time points of infection. Each time point represents the geometric mean of the CFU from four or five animals. The statistical significance of the decrease in bacterial load in anti-IL-10R mAb-treated mice compared with control mice is labeled with asterisks (**, $p < 0.01$). B, IFN- γ levels in sera and homogenates of spleens and lungs of four or five infected mice per group at different time points of infection. Values from noninfected animals were below the detection limit of the assay and were not represented. Data are represented as the means $\pm$ 1 SD of the mean. Values for anti-IL-10R mAb-treated mice were statistically different from control mice at the time points labeled with asterisks. *, $p < 0.05$; **, $p < 0.01$.

Thus, we infected mice with *M. avium* and treated them with either anti-IL-10R mAb or nonimmune rat IgG as control for 20 days. The animals were then sacrificed, and CD4 $^+$ T cell function as well as macrophage activation were studied. As shown in Fig. 2A, CD4 $^+$ T cells from spleens of mice treated with anti-IL-10R mAb produced significantly increased amounts of IFN- γ in response to specific mycobacterial proteins when compared with cells from control-infected animals. Proliferative responses to *M. avium* proteins were identical for CD4 $^+$ T cells from spleens of both groups

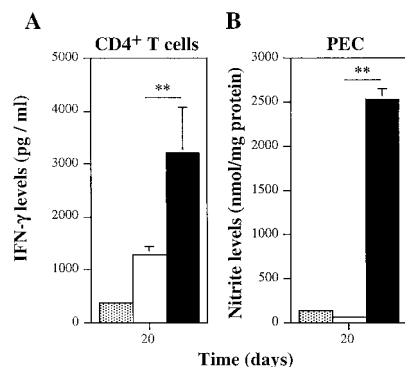


FIGURE 2. Effect of anti-IL-10R mAb administration on the ability of both purified CD4⁺ spleen cells to produce IFN-γ (A) and PEC to secrete nitrite (B) in response to *M. avium*-specific Ags and LPS, respectively. BALB/c mice were infected i.v. with 10⁶ CFU of *M. avium* for 20 days and were treated i.p. with anti-IL-10R mAb (■) or rat IgG (□) every other day throughout the experimental period of infection. A, At 20 days of infection, CD4⁺ T cells from spleens of uninfected mice (▨) or infected mice treated (■) or not treated (□) with anti-IL-10R mAb were purified, as described in *Materials and Methods*, and cultured in the presence of different stimuli or medium alone. After 72 h, supernatants of cell cultures were harvested, and the amount of IFN-γ present in supernatants was measured by ELISA. IFN-γ levels in supernatants of nonstimulated cell cultures were below the detection limit of the assay and were not represented. Values are reported as means ± 1 SD of triplicates of pool of CD4⁺ T cells from four individual mice for each group (*n* = 4 mice). B, PEC from uninfected mice (▨) and infected mice treated (■) or not treated (□) with anti-IL-10R mAb were collected at 20 days of infection, cultured, and used to assess their ability to secrete nitrite in response to LPS. Data are reported as mean ± 1 SD of triplicates from four individual mice for each group. Statistical analysis was performed using the Student's *t* test, and significant differences between anti-IL-10R mAb vs nonimmune rat IgG treatments are shown. *, *p* < 0.05; **, *p* < 0.01.

of infected mice (data not shown). FACS analysis showed that the number of CD4⁺ T cells in culture was similar for all groups of mice (9.5×10^4 cells for noninfected mice; 9.4×10^4 cells for anti-IL-10R mAb-treated infected mice; and 9.4×10^4 cells for control-infected mice). These data suggest that the increment in the resistance to *M. avium* infection as a result of blocking the IL-10R in the early phase of the infection could be at least partially due to the increased priming of CD4⁺ T cells for IFN-γ secretion.

As shown in Fig. 2B, treatment of infected mice with anti-IL-10R mAb also increased the activation of macrophages explanted from the peritoneal cavity of infected mice. Peritoneal macrophages from infected mice treated with anti-IL-10R mAb produced 40-fold higher amounts of nitrite than those from infected control animals. These data may indicate that treatment with anti-IL-10R mAb in the early phase of infection augmented macrophage functions other than the production of reactive nitrogen intermediates, namely those involved in the mechanisms of resistance to *M. avium* infection.

Anti-IL-10R mAbs improve the efficacy of chemotherapy in refractory MAC infections

Given the effects of anti-IL-10R treatment on macrophage activation, we analyzed whether this treatment had a synergistic effect on antimicrobial therapy. BALB/c mice were infected with *M. avium*, and half of them were given a combination of antibiotics in one of two regimens. In the first, chemotherapy was started at the beginning of infection (Fig. 3A), whereas in a second protocol, chemotherapy started only after 1 mo of infection (Fig. 3B). The antibiotics were given in combination either with control IgG1 from

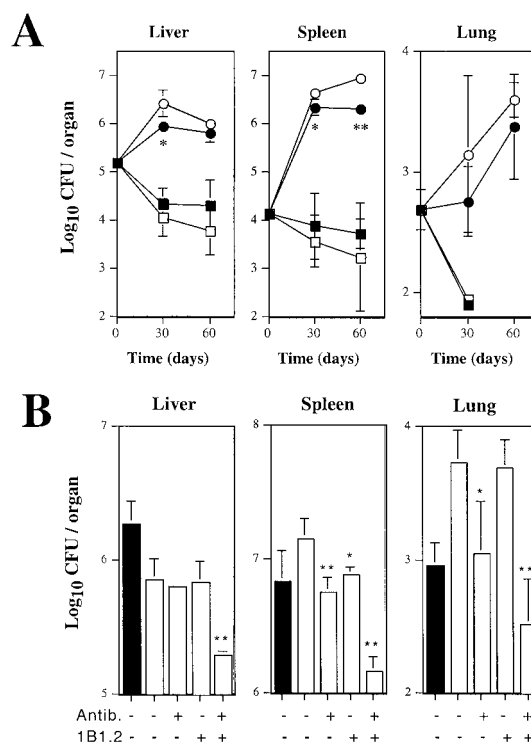


FIGURE 3. Evaluation of the effect of blocking the IL-10R during a three-drug regimen on mycobacterial clearance in BALB/c mice infected with *M. avium*. A, Mice were infected i.v. with 10⁶ CFU of *M. avium* 2447 and treated with rifabutin-clarithromycin-ethambutol in the drinking water during the whole experimental period of infection (□, ■). As control of antibiotic therapy, mice drank nonsupplemented water (○, ●). During the period of the antibiotic regimen, mice were injected i.p. every other day with anti-IL-10R mAb (■, ●) or with an irrelevant mAb (□, ○). B, Mice were infected i.v. with 10⁶ CFU of *M. avium* 2447 and treated with rifabutin-clarithromycin-ethambutol (Antib.) in the drinking water starting 30 days after the infection or given no antibiotics throughout the experimental period of infection. During the period of the antibiotic regimen, mice were injected i.p. every other day with anti-IL-10R mAb (1B1.2) or with an irrelevant mAb. Mycobacterial loads were determined on day 30 (■) or day 60 (□). The data are represented as the mean of the log₁₀ number of CFU recovered per organ ± 1 SD of the mean (*n* = 4 or 5 animals). Statistical analysis was done according to the Student's *t* test by comparing the groups that received anti-IL-10R Abs with the respective control (*top panels*) or between the loads at day 60 in mice receiving neither antibiotics nor anti-IL-10R mAb and the other groups. Statistical significant decreases in bacterial load: *, *p* < 0.05; **, *p* < 0.01.

hybridoma GL113 or with anti-IL-10R mAb. As shown in Fig. 3A, chemotherapy alone was effective when started early in infection. In that situation, anti-IL-10R mAb therapy did not improve the effectiveness of chemotherapy (Fig. 3A). When antibiotic therapy was started 1 mo after the inoculation of the mycobacteria, it was without any effect in the liver and had a minor effect in the spleen and lung (Fig. 3B). As previously observed, treatment of mice with anti-IL-10R alone starting at 1 mo of infection had also no effect in the liver and lung and caused a very small decrease in the spleen. However, the treatment with anti-IL-10R Abs reverted the refractoriness to chemotherapy of these chronically infected animals. Indeed, mice under this combined therapy showed a significant reduction in bacterial loads in the lung (1.21 log₁₀), in the spleen (0.97 log₁₀), and in the liver (0.56 log₁₀) as compared with mice treated with GL113 mAb without chemotherapy. Additionally, the combined therapy was effective in the clearance of bacteria in both liver and spleen when compared with bacterial loads

found in mice at 30 days of infection, the starting point for the treatment (liver $p = 0.000$ and spleen $p = 0.003$).

Anti-IL-10R mAbs can substitute for adjuvants in subunit MAC vaccines

Since we found that anti-IL-10R treatment would enhance the priming of presumably protective, IFN- γ -secreting CD4⁺ T cells, we evaluated the usefulness of the anti-IL-10R mAb administration on the design of antimycobacterial vaccines. We used secreted proteins from *M. avium* as the Ag source. This choice was based on previous studies from our lab that showed the potential of CFP in the induction of a protective immunity to *M. avium* (14). Furthermore, the protection against *M. avium* infection conferred by the subunit vaccine composed of CFP emulsified in an adjuvant had the same magnitude as that conferred by BCG (15). Mice were immunized with CFPs either alone or admixed with DDA as an adjuvant. As controls for the nonspecific inflammatory-dependent induction of resistance to infection, mice were injected with DDA or PBS. To test whether blocking the IL-10R during the immunization had an effect on the resistance provided by the subunit vaccines, mice were injected with anti-IL-10R mAb or with rat non-immune IgG as control 2 h before every dose of the subunit vaccines. As shown in Fig. 4, the combination of CFP and DDA induced significant protection in mice against a challenge with the homologous *M. avium* strain. CFP alone, on the other hand, failed to protect mice. However, treatment of mice with anti-IL-10R mAb during vaccination made CFP alone immunogenic, leading to the induction of protective immunity. The treatment with the anti-IL-10R mAb did not improve the efficacy of the vaccine consisting of CFP plus DDA (Fig. 4).

Discussion

Studies testing the effectiveness of anti-IL-10R mAb on its ability to neutralize the activity of IL-10 have already been performed either in vitro (37) or in vivo (42). Mice infected i.v. with a lethal inoculum dose of *Listeria monocytogenes* were rescued from death by the administration of anti-IL-10R during experimental infection (42). However, the present results show that interfering with the activity of IL-10 during an infection by *M. avium* had a small impact on the course of infection (Figs. 1 and 3). These data contrast with previously published experiments in which anti-IL-10 Abs were used and which described major effects of the treatment

on the course of infection (24, 25). These differences may be related to the different strains of *M. avium* used (24, 25), the differences in the route of infection (24), or the strains of mice studied (24). In our hands, the anti-IL-10R Abs had little impact on the outcome of infection, but still led to significant differences in mycobacterial loads. Previous work from our laboratory, using anti-IL-10 mAbs, failed to show any effects on the course of infection (43). Also, we have recently found that IL-10 gene-deficient mice were only marginally more resistant to infection by the strain of *M. avium* used in this study than control mice (44), mimicking the data reported in this work. Overall, we are confident to state that IL-10 plays a minor role in determining susceptibility to infection by *M. avium*, although some protection may be found at early stages of infection by blocking its activity. These data are similar to those obtained with experimental therapies with rIL-12 in which the immunomodulatory interventions accelerated the immune response by increasing the levels of IFN- γ in early phases of infection (14), but did not dramatically affect the overall outcome of the infection (14, 45).

IL-10 has been described in the literature as a potent macrophage-deactivating factor by suppressing activation of macrophages and dendritic cells, inhibiting their ability to both secrete cytokines and express costimulatory molecules at their surface (17–19). In our study, we found that the minor protection afforded by treatment with anti-IL-10R mAb observed in the early phase of *M. avium* infection was associated with an increase in both the priming of CD4⁺ T cells to produce IFN- γ and activation of some functions of the macrophages (Fig. 2). Indeed, IFN- γ is important in an early phase of the infection for the induction of protective immune mechanisms that lead to mycobacteriostasis (14, 40, 41). Later, the increment in IFN- γ production induced by anti-IL-10R mAb treatment did not improve the resistance to *M. avium*, in agreement with our previous work in which neutralization of IFN- γ after 30 days of infection had a minor effect on bacterial loads (41). Thus, the effects of the treatment with anti-IL-10R mAb on the course of a primary infection may relate to a combination of the effects on the emergence of protective T cells producing IFN- γ and the promotion of the macrophage activation.

Our data highlight the potential of an anti-IL-10R treatment to revert the refractoriness of the infections by MAC to chemotherapy. We observed that once the infection by *M. avium* was established, a combination of antibiotics, which was effective

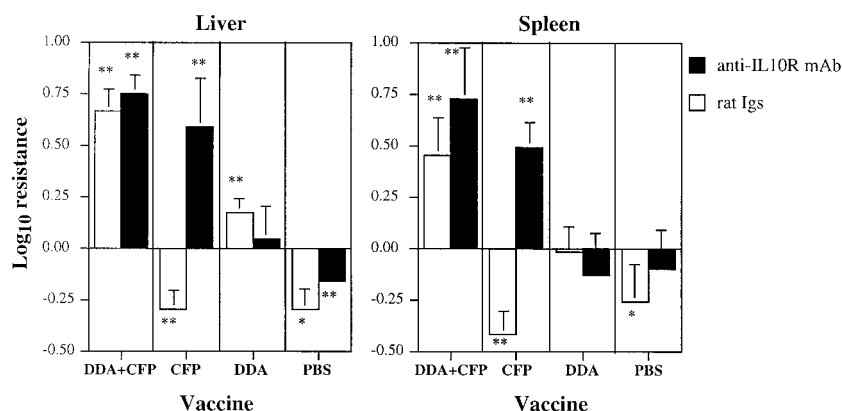


FIGURE 4. Effect of anti-IL-10R mAb administration during the immunization regimen on the protection provided by experimental vaccines against *M. avium* infection. Mice were immunized with CFP either alone or emulsified with DDA. As controls of immunization, mice were injected with DDA or PBS. Two hours before each immunization, mice were treated with anti-IL-10R mAb (■) or nonimmune rat IgG as control (□). Animals were challenged i.v. with *M. avium* 2447 at 2 mo after the last immunization. At the same time, a group of untreated mice was infected and viable bacteria were quantified 30 days later. Results are shown as log₁₀ protection, which was calculated by subtracting the mean log₁₀ CFU in the treated group from the mean log₁₀ CFU in untreated control group. Statistically significant protection between treated and untreated mice: *, $p < 0.05$; **, $p < 0.01$.

when administered early in infection, became unable to clear the infection. This is a situation that corresponds more closely to the clinical situation in which patients are diagnosed when already heavily infected by nontuberculous mycobacteria (3). However, when antibiotic chemotherapy was associated with anti-IL-10R treatments, the effectiveness of the therapy was regained in those chronic infections. This contrasted with the lack of effects of the mAb therapy when chemotherapy was started at the time of infection. Thus, the combination of therapeutic strategies aimed at interfering with IL-10 signaling with classical chemotherapy may prove of importance in the management of human infections by MAC. In this regard, published data suggest that increased IL-10 production may contribute to a predisposition to *M. avium* infections in AIDS patients (46–48). It would also be of interest to study such an approach in the therapy of tuberculosis, as it was shown that immune cells from tuberculosis patients produce IL-10 and that anergy in tuberculosis patients was related to T cell-derived IL-10 (49–51). Curiously, similar to our present findings, IL-10 seems not to underlie the susceptibility to tuberculosis in the mouse model (52). Since long-term treatment of both tuberculosis and *M. avium* infection with the appropriate conventional chemotherapy usually leads to severe side effects (11, 12), the possibility of improving antimycobacterial activity of current antibiotics by interfering with IL-10 signaling may help circumvent these problems.

The mechanisms underlying the effects of the anti-IL-10R treatment on chemotherapy are still unknown, but it is likely that IL-10 being produced at the peak of the immune response blocks the activity of the antibiotics by deactivating the macrophage. Indeed, the effects of anti-IL-10R treatment on macrophage functions are more pronounced than those on T cell activity. How IL-10 interferes in the intramacrophagic action of antibiotics is currently being studied. On the other hand, the data obtained in this study with anti-IL-10R mAbs are reminiscent of the data obtained by Doherty and Sher (45), with the association of chemotherapy with rIL-12 therapy. In that work, a synergistic activity was found between the cytokine and the antimicrobial compounds, which was dependent on IFN- γ production. On the other hand, clinical trials have found that IFN- γ may improve the antibiotic chemotherapy of MAC infections (53). Thus, it is possible that the synergistic activity of anti-IL-10R mAb and antibiotic therapy may depend on both an increased IFN- γ production and an enhanced response by the macrophage to this cytokine in a cytokine cascade most likely also involving increased IL-12 production.

The interference with IL-10 may also prove of interest in the design of new vaccines, namely those using subunit components of pathogens. Our data showed that anti-IL-10R mAb could substitute for the adjuvant in the induction of protective immunity. However, it should be stressed that much more research in this area should be done to ascertain the safety of such strategies. The mechanism of action is unclear, but it may be related to the one described by Castro et al. (36). In their system, LPS was required in order for soluble protein Ag to induce a Th1 response when IL-10 activity was abrogated. Our Ag preparations include not only proteins, but probably other mycobacterial components present in trace amounts, such as glycolipids, that could be recognized by macrophages and/or dendritic cells through the pattern-recognition receptors, eliciting both a proinflammatory immune response (54–57) and dendritic cell maturation (58). Thus, neutralization of IL-10 may augment the adjuvant effect of these contaminants in the subunit vaccine. The adjuvant effects of anti-IL-10R mAb may relate to the increased expression of costimulatory molecules and class II MHC (17–19) as well as to effects on APC, such as den-

dritic cells, since the biology of the latter is strongly influenced by IL-10 (58–60).

In conclusion, although treatments with anti-IL-10R mAb alone may not improve the outcome of mycobacterial infections, they may be useful in combination with antibiotic chemotherapy as well as in the field of vaccination as adjuvants in subunit vaccines.

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References

- Schlager, N. W., T. J. Harkin, and W. N. Rom. 1996. Principles of therapy of tuberculosis in the modern era. In *Tuberculosis*. W. N. Rom and S. M. Garay, eds. Little, Brown and Company, Boston, p. 751.
- Harkin, T. J., and H. W. Harris. 1996. Treatment of multidrug-resistant tuberculosis. In *Tuberculosis*. W. N. Rom and S. M. Garay, eds. Little, Brown and Company, Boston, p. 843.
- Inderlied, C. B., C. A. Kemper, and L. E. Bermudez. 1993. The *Mycobacterium avium* complex. *Clin. Microbiol. Rev.* 6:266.
- Dautzenberg, B., C. Truffot, S. Legris, M. C. Meyohas, H. C. Berlie, A. Mercat, S. Chevret, and J. Grosset. 1991. Activity of clarithromycin against *Mycobacterium avium* infection in patients with the acquired immune deficiency syndrome: a controlled clinical trial. *Am. Rev. Respir. Dis.* 144:564.
- Fernandes, P. B., D. J. Hardy, D. McDaniel, C. W. Hanson, and R. N. Swanson. 1989. In vitro and in vivo activities of clarithromycin against *Mycobacterium avium*. *Antimicrob. Agents Chemother.* 33:1531.
- Bermudez, L. E., M. Petrofsky, P. Kolonoski, and L. S. Young. 1998. Emergence of *Mycobacterium avium* populations resistant to macrolides during experimental chemotherapy. *Antimicrob. Agents Chemother.* 42:180.
- Ji, B., N. Lounis, C. Truffot-Pernot, and J. Grosset. 1992. Selection of resistant mutants of *Mycobacterium avium* in beige mice by clarithromycin monotherapy. *Antimicrob. Agents Chemother.* 36:2839.
- Pierce, M., S. Crampton, D. Henry, L. Heifets, A. LaMarca, M. Montecalvo, G. P. Wormser, H. Jablonowski, J. Jemsek, M. Cynamon, et al. 1996. A randomized trial of clarithromycin as prophylaxis against disseminated *Mycobacterium avium* complex infection in patients with advanced acquired immunodeficiency syndrome. *N. Engl. J. Med.* 335:384.
- Bermudez, L. E., K. A. Nash, M. Petrofsky, L. S. Young, and C. B. Inderlied. 1996. Effect of ethambutol on emergence of clarithromycin-resistant *Mycobacterium avium* complex in the beige mouse model. *J. Infect. Dis.* 174:1218.
- Gordin, F. M., P. M. Sullam, S. D. Shafran, D. L. Cohn, B. Wynne, L. Paxton, K. Perry, and C. R. Horsburgh, Jr. 1999. A randomized, placebo-controlled study of rifabutin added to a regimen of clarithromycin and ethambutol for treatment of disseminated infection with *Mycobacterium avium* complex. *Clin. Infect. Dis.* 28:1080.
- Shafran, S. D., J. Deschenes, M. Miller, P. Phillips, and E. Toma. 1994. Uveitis and pseudojaundice during a regimen of clarithromycin, rifabutin, and ethambutol: MAC Study Group of the Canadian HIV Trials Network. *N. Engl. J. Med.* 330:438.
- Shafran, S. D., J. Singer, D. P. Zarowny, P. Phillips, I. Salit, S. L. Walmsley, I. W. Fong, M. J. Gill, A. R. Rachlis, R. G. Lalonde, et al. 1996. A comparison of two regimens for the treatment of *Mycobacterium avium* complex bacteremia in AIDS: rifabutin, ethambutol, and clarithromycin versus rifampin, ethambutol, clofazimine, and ciprofloxacin: Canadian HIV Trials Network Protocol 010 Study Group. *N. Engl. J. Med.* 335:377.
- Fine, P. E. 1988. BCG vaccination against tuberculosis and leprosy. *Br. Med. Bull.* 44:691.
- Silva, R. A., T. F. Pais, and R. Appelberg. 1998. Evaluation of IL-12 in immunotherapy and vaccine design in experimental *Mycobacterium avium* infections. *J. Immunol.* 161:5578.
- Silva, R. A., T. F. Pais, and R. Appelberg. 2000. Effects of interleukin-12 in the long-term protection conferred by a *Mycobacterium avium* subunit vaccine. *Scand. J. Immunol.* 52:531.
- Leal, I. S., B. Smedegard, P. Andersen, and R. Appelberg. 1999. Interleukin-6 and interleukin-12 participate in induction of a type 1 protective T-cell response during vaccination with a tuberculosis subunit vaccine. *Infect. Immun.* 67:5747.
- Moore, K. W., A. O'Garra, R. de Waal Malefyt, P. Vieira, and T. R. Mosmann. 1993. Interleukin-10. *Annu. Rev. Immunol.* 11:165.
- De Vries, J. E. 1995. Immunosuppressive and anti-inflammatory properties of interleukin 10. *Ann. Med.* 27:537.
- Moore, K. W., R. W. Malefyt, R. L. Coffman, and A. O'Garra. 2001. Interleukin-10 and the interleukin-10 receptor. *Annu. Rev. Immunol.* 19:683.
- Reed, S. G., C. E. Brownell, D. M. Russo, J. S. Silva, K. H. Grabstein, and P. J. Morrissey. 1994. IL-10 mediates susceptibility to *Trypanosoma cruzi* infection. *J. Immunol.* 153:3135.
- Wagner, R. D., N. M. Maroushek, J. F. Brown, and C. J. Czuprynski. 1994. Treatment with anti-interleukin-10 monoclonal antibody enhances early resistance to but impairs complete clearance of *Listeria monocytogenes* infection in mice. *Infect. Immun.* 62:2345.
- Dai, W. J., G. Kohler, and F. Brombacher. 1997. Both innate and acquired immunity to *Listeria monocytogenes* infection are increased in IL-10-deficient mice. *J. Immunol.* 158:2259.

23. Genovese, F., G. Mancuso, M. Cuzzola, C. Biondo, C. Beninati, D. Delfino, and G. Teti. 1999. Role of IL-10 in a neonatal mouse listeriosis model. *J. Immunol.* 163:2777.
24. Bermudez, L. E., and J. Champs. 1993. Infection with *Mycobacterium avium* induces production of interleukin-10 (IL-10), and administration of anti-IL-10 antibody is associated with enhanced resistance to infection in mice. *Infect. Immun.* 61:3093.
25. Denis, M., and E. Ghadirian. 1993. IL-10 neutralization augments mouse resistance to systemic *Mycobacterium avium* infections. *J. Immunol.* 151:5425.
26. Jacobs, M., N. Brown, N. Allie, R. Gulert, and B. Ryffel. 2000. Increased resistance to mycobacterial infection in the absence of interleukin-10. *Immunology* 100:494.
27. Murray, P. J., and R. A. Young. 1999. Increased antimycobacterial immunity in interleukin-10-deficient mice. *Infect. Immun.* 67:3087.
28. Bogdan, C., Y. Vodovotz, and C. Nathan. 1991. Macrophage deactivation by interleukin 10. *J. Exp. Med.* 174:1549.
29. Gazzinelli, R. T., I. P. Oswald, S. L. James, and A. Sher. 1992. IL-10 inhibits parasite killing and nitrogen oxide production by IFN- γ -activated macrophages. *J. Immunol.* 148:1792.
30. Malefyt, R. W., J. Abrams, B. Bennett, C. G. Figdor, and J. E. de Vries. 1991. Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J. Exp. Med.* 174:1209.
31. Oswald, I. P., T. A. Wynn, A. Sher, and S. L. James. 1992. Interleukin 10 inhibits macrophage microbicidal activity by blocking the endogenous production of tumor necrosis factor α required as a costimulatory factor for interferon γ -induced activation. *Proc. Natl. Acad. Sci. USA* 89:8676.
32. Ding, L., and E. M. Shevach. 1992. IL-10 inhibits mitogen-induced T cell proliferation by selectively inhibiting macrophage costimulatory function. *J. Immunol.* 148:3133.
33. Willems, F., A. Marchant, J. P. Delville, C. Gerard, A. Delvaux, T. Velu, M. de Boer, and M. Goldman. 1994. Interleukin-10 inhibits B7 and intercellular adhesion molecule-1 expression on human monocytes. *Eur. J. Immunol.* 24:1007.
34. Malefyt, R. W., J. Haanen, H. Spits, M. G. Roncarolo, A. te Velde, C. Figdor, K. Johnson, R. Kastelein, H. Yssel, and J. E. de Vries. 1991. Interleukin 10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminishing the antigen-presenting capacity of monocytes via down-regulation of class II major histocompatibility complex expression. *J. Exp. Med.* 174:915.
35. Koppelman, B., J. J. Neefjes, J. E. de Vries, and R. W. Malefyt. 1997. Interleukin-10 down-regulates MHC class II $\alpha\beta$ peptide complexes at the plasma membrane of monocytes by affecting arrival and recycling. *Immunity* 7:861.
36. Castro, A. G., M. Neighbors, S. D. Hurst, F. Zonin, R. A. Silva, E. Murphy, Y. J. Liu, and A. O'Garra. 2000. Anti-interleukin 10 receptor monoclonal antibody is an adjuvant for T helper cell type 1 responses to soluble antigen only in the presence of lipopolysaccharide. *J. Exp. Med.* 192:1529.
37. O'Farrell, A. M., Y. Liu, K. W. Moore, and A. L. Mui. 1998. IL-10 inhibits macrophage activation and proliferation by distinct signaling mechanisms: evidence for Stat3-dependent and -independent pathways. *EMBO J.* 17:1006.
38. Pais, T. F., J. F. Cunha, and R. Appelberg. 2000. Antigen specificity of T-cell response to *Mycobacterium avium* infection in mice. *Infect. Immun.* 68:4805.
39. Appelberg, R., I. M. Orme, M. I. Pinto de Sousa, and M. T. Silva. 1992. In vitro effects of interleukin-4 on interferon- γ -induced macrophage activation. *Immunology* 76:553.
40. Silva, R. A., M. Flórido, and R. Appelberg. 2001. Interleukin-12 primes CD4⁺ T cells for interferon- γ production and protective immunity during *Mycobacterium avium* infection. *Immunology. In press.*
41. Appelberg, R., A. G. Castro, J. Pedrosa, R. A. Silva, I. M. Orme, and P. Minoprio. 1994. Role of γ interferon and tumor necrosis factor α during T-cell-independent and -dependent phases of *Mycobacterium avium* infection. *Infect. Immun.* 62:3962.
42. Silva, R. A., and R. Appelberg. 2001. Blocking the receptor for interleukin 10 protects mice from lethal listeriosis. *Antimicrob. Agents Chemother.* 45:1312.
43. Appelberg, R., A. G. Castro, R. A. Silva, and J. Pedrosa. 1997. Cytokines involved in resistance to *Mycobacterium avium* in a mouse model of infection. *Med. Princ. Practice* 6:97.
44. Gonçalves, A. S., and R. Appelberg. 2001. Effects of recombinant granulocyte-colony stimulating factor administration during *Mycobacterium avium* infection in mice. *Clin. Exp. Immunol. In press.*
45. Doherty, T. M., and A. Sher. 1998. IL-12 promotes drug-induced clearance of *Mycobacterium avium* infection in mice. *J. Immunol.* 160:5428.
46. Clerici, M., T. A. Wynn, J. A. Berzofsky, S. P. Blatt, C. W. Hendrix, A. Sher, R. L. Coffman, and G. M. Shearer. 1994. Role of interleukin-10 in T helper cell dysfunction in asymptomatic individuals infected with the human immunodeficiency virus. *J. Clin. Invest.* 93:768.
47. Muller, F., P. Aukrust, E. Lien, C. J. Haug, and S. S. Froland. 1998. Enhanced interleukin-10 production in response to *Mycobacterium avium* products in mononuclear cells from patients with human immunodeficiency virus infection. *J. Infect. Dis.* 177:586.
48. Stylianou, E., P. Aukrust, D. Kvale, F. Muller, and S. S. Froland. 1999. IL-10 in HIV infection: increasing serum IL-10 levels with disease progression: down-regulatory effect of potent anti-retroviral therapy. *Clin. Exp. Immunol.* 116:115.
49. Boussiotis, V. A., E. Y. Tsai, E. J. Yunis, S. Shim, J. C. Delgado, C. C. Dascher, A. Berezhovskaya, D. Rousset, J. M. Reynes, and A. E. Goldfeld. 2000. IL-10-producing T cells suppress immune responses in anergic tuberculosis patients. *J. Clin. Invest.* 105:1317.
50. Zhang, M., J. Gong, D. V. Iyer, B. E. Jones, R. L. Modlin, and P. F. Barnes. 1994. T cell cytokine responses in persons with tuberculosis and human immunodeficiency virus infection. *J. Clin. Invest.* 94:2435.
51. Gerosa, F., C. Nisii, S. Righetti, R. Micciolo, M. Marchesini, A. Cazzadori, and G. Trinchieri. 1999. CD4⁺ T cell clones producing both interferon- γ and interleukin-10 predominate in bronchoalveolar lavages of active pulmonary tuberculosis patients. *Clin. Immunol.* 92:224.
52. North, R. J. 1998. Mice incapable of making IL-4 or IL-10 display normal resistance to infection with *Mycobacterium tuberculosis*. *Clin. Exp. Immunol.* 113:55.
53. Holland, S. M., E. M. Eisenstein, D. B. Kuhns, M. L. Turner, T. A. Fleisher, W. Strober, and J. I. Gallin. 1994. Treatment of refractory disseminated nontuberculous mycobacterial infection with interferon γ . *N. Engl. J. Med.* 330:1348.
54. Underhill, D. M., A. Ozinsky, K. D. Smith, and A. Aderem. 1999. Toll-like receptor-2 mediates mycobacteria-induced proinflammatory signaling in macrophages. *Proc. Natl. Acad. Sci. USA* 96:14459.
55. Means, T. K., S. Wang, E. Lien, A. Yoshimura, D. T. Golenbock, and M. J. Fenton. 1999. Human Toll-like receptors mediate cellular activation by *Mycobacterium tuberculosis*. *J. Immunol.* 163:3920.
56. Aderem, A., and R. J. Ulevitch. 2000. Toll-like receptors in the induction of the innate immune response. *Nature* 406:782.
57. Hertz, C., S. Kiertcher, P. Godowski, D. Bouis, M. Norgard, M. Roth, and R. Modlin. 2001. Microbial lipopeptides stimulate dendritic cell maturation via Toll-like receptor 2. *J. Immunol.* 166:2444.
58. Steinbrink, K., M. Wolff, H. Jonuleit, J. Knop, and A. H. Enk. 1997. Induction of tolerance by IL-10-treated dendritic cells. *J. Immunol.* 159:4772.
59. Igietsme, J. U., G. A. Ananaba, J. Bolier, S. Bowers, T. Moore, T. Belay, F. O. Eko, D. Lyn, and C. M. Black. 2000. Suppression of endogenous IL-10 gene expression in dendritic cells enhances antigen presentation for specific Th1 induction: potential for cellular vaccine development. *J. Immunol.* 164:4212.
60. Corinti, S., C. Albanesi, A. la Sala, S. Pastore, and G. Girolomoni. 2001. Regulatory activity of autocrine IL-10 on dendritic cell functions. *J. Immunol.* 166:4312.