Effect of Rifabutin on Disseminated Mycobacterium avium Infections in Thymectomized, CD4 T-Cell-Deficient Mice

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Disseminated Mycobacterium avium infection is the major cause of bacteremia in patients with acquired immunodeficiency syndrome. We present here a new animal model of this disease, thymectomized C57BL/6 mice that were intravenously infused with monoclonal antibody to selectively deplete CD4+ T cells. The increased susceptibility of such animals to M. avium infection is comparable to that of C57BL/6 beige mice and thus may provide a viable alternative to the latter model. Further, using representative strains of acquired immunodeficiency syndrome-associated M. avium (serotypes 1, 4, and 8 and a rough isolate), we show that the course of such infections in thymectomized, CD4-deficient mice can be markedly restrained and in some cases the infections can be sterilized by treatment over a 120-day period with a regimen containing 40 mg of the new antimycobacterial agent rifabutin per kg (body weight).

Once a fairly uncommon occurrence, infections caused by members of the Mycobacterium avium complex have increased enormously over the past few years as a result of the acquired immunodeficiency syndrome (AIDS) epidemic to the extent that they now represent the most common form of bacteremia in patients with AIDS (1, 16). Chemotherapy of such infections has proved difficult not only because of resistance of these organisms to conventional antimycobacterial therapy but because diagnosis is usually made only when bacterial numbers reach very high, potentially untreatable, levels (16).

We report here a new animal model of M. avium infections, mice specifically depleted of CD4+ T cells in an attempt to model the specific immunological defect in AIDS. Such mice are significantly more susceptible to M. avium infections than control mice and thus may provide a more immunologically accurate alternative to the C57BL/6 beige mouse model (5) in the sense that they mimic AIDS in terms of specific CD4 T-cell-mediated immune depression while beige mice possess biochemical lesions at the level of intracellular bacterial killing, similar to Chediak-Higashi syndrome (11). In addition, we show that treatment of infected, thymectomized, CD4-deficient (TxCD4+) mice with the new antimycobacterial drug rifabutin substantially reduced the growth of and in some cases sterilized three of four M. avium isolates tested.

MATERIALS AND METHODS

Mice. Specific-pathogen-free female C57BL/6J and C57BL/6J-bg/bg (beige) mice were purchased from the Jackson Laboratory, Bar Harbor, Maine, and maintained under isolated barrier conditions in our facility. Because of their poor natural killer cell activity (11–13), we routinely autopsied beige mice at each harvest time point for evidence of tumor growth; data from 16% of the mice in our colony harvested at the last time points (i.e., when they were about 6 months of age) were excluded for this reason.

One group of C57BL/6 mice were thymectomized at 4 weeks of age, followed 1 week later by intravenous infusion with 200 μg of purified monoclonal GK1.5 (anti-CD4) antibody (3) in 200 μl of sterile saline. Antibody was purified from ascitic or tissue culture medium by passage through a Gamma-G column (Genex Corp., Gaithersburg, Md.), followed by dialysis and lyophilization. Changes in T-cell numbers in these mice and in thymectomized controls were monitored by flow cytometry. At indicated times, spleen cells from two mice were harvested, pooled, and incubated on ice with a 1:50 dilution of GK1.5 monoclonal antibody. Cells were then washed and stained with goat anti-rat fluorescein isothiocyanate-labeled antibody, followed by examination with a Coulter Epics V flow cytometer.

Bacteria. Four isolates of M. avium from AIDS patients selected for serotypes representative of those most commonly seen in AIDS patients but otherwise chosen at random were obtained from the Mycobacteria Typing Laboratory, National Jewish Center, Denver, Colo. The strains selected were 5-74 (serotype 8), 6-12 (serotype 1), 6-13 (serotype 4), and 6-28 (untypeable; rough morphology). Serotypes were verified by using monoclonal antibodies to the type-specific glycopolipid antigens (15). The isolates were grown in Proskauer-Beck nutrient medium and harvested in mid-log phase. Bacteria were frozen at −70°C and then thawed and diluted appropriately in sterile saline prior to being injected in an inoculum of 200 μl of saline via a lateral tail vein. In each experiment, a dose of approximately 10⁴ viable organisms was administered when the mice were 8 weeks of age.

Course of infection. The course of each infection was monitored over time by plating serial dilutions of individual whole-organ homogenates on 7H11 agar and counting bacterial colonies after 14 to 20 days of incubation at 37°C in humidified air. The limits of detection for this plating method are approximately 50 organisms in the spleen and 100 in the liver and lungs (CFU per organ). Bone marrow counts represent mycobacteria harvested from both femurs following repeated flushing with sterile phosphate-buffered saline.

Drugs. Ethambutol (Lederle Laboratories, Pearl River, N.Y.) and rifabutin (Adria Laboratories, Columbus, Ohio) were supplied by the manufacturers. Rifampin was purchased from Sigma Chemical Co. (St. Louis, Mo.). Drug therapies were initiated 20 days postinfection.

Because the long treatment regimens precluded the use of gavage, the mice were exposed to drugs in their drinking
water, which was available ad libitum. Daily water consumption was regularly checked, and drug concentrations were adjusted to provide the correct dosage per day for each animal. We did not observe more than 10% variability in water uptake by the different groups. Dosages administered were as follows: rifabutin and rifampin, 40 mg/kg (body weight) per day; and ethambutol, 25 mg/kg per day. Drug-containing water bottles were replaced each 48 to 72 h; all agents appeared to be stable over this period and retained their activity in terms of MICs against a drug-susceptible strain of Mycobacterium tuberculosis. Thirty days after initiation of treatment, levels of ethambutol and rifampin in serum were 4 and 21 μg/ml, respectively, as determined by bioassay (8). Rifabutin in serum could not be accurately detected by these methods, but a previously described (9) high-pressure liquid chromatography method gave values of 0.18 μg/ml of serum for this agent.

MIC determinations. MICs of the three drugs used were determined by counting bacterial colonies on oleate-albumin-dextrose-catalase (OADC)-supplemented 7H11 agar further supplemented with each compound to give final drug concentrations of 0.15 to 20 μg/ml. Dilutions of bacteria from 10⁴ to 10³ CFU were inoculated onto each plate quadrant in volumes of 200 μl. The isolate was considered susceptible to a given drug concentration if no colony growth was observed over a 21-day period.

RESULTS

Depletion of CD4⁺ T cells from C57BL/6 mice. As shown in Fig. 1, infusion of thymectomized mice with monoclonal GK1.5 antibody resulted in a very dramatic fall in numbers of splenic T cells staining for the CD4 marker. Moreover, because the lack of a thymus precluded regeneration, these levels remained below 10% for the remainder of this experiment.

FIG. 1. Immunoﬂuorescence analysis of CD4⁺ T-cell depletion within murine spleen cells following intravenous administration (on day 0) of monoclonal GK1.5 antibody. Flow cytometry analysis was performed on pooled cells from two animals; the data shown are means of three determinations. Mice tested were thymectomized only (■) or thymectomized plus antibody treated (□). CD4⁺ T-cell numbers were found to be equivalent in normal and sham-thymectomized C57BL/6 and C57BL/6 beige mice (data not shown).

Growth of M. avium in beige mice and in TxCD4⁻ mice. To date, studies of M. avium infection in beige mice have almost exclusively used isolates (no. 571-8; no. 101) of relatively high virulence (in that they are usually fatal in the mouse infection model). Since most M. avium isolates tend to be of only low or moderate virulence (in that they persist or grow slowly in but are not fatal to C57BL/6 mice), we used strain 5-74 to compare the beige and TxCD4⁻ models. As can be seen in Fig. 2, this organism grows, but slowly, in C57BL/6 mice.

It was found (Fig. 2) that both mouse models were clearly more susceptible to the infection than were normal, immunocompetent controls. While growths of the M. avium infection were equivalent in the spleens of the beige and TxCD4⁻ mice, the beige mice were clearly more susceptible to infection in the lungs (P < 0.02). On the other hand, the TxCD4⁻ mice showed evidence of chronic disease in the liver and accelerated dissemination of the infection to the bone marrow (P < 0.05).

Chemotherapy of M. avium infections in TxCD4⁻ mice. The TxCD4⁻ mouse model was used to test the activities of rifampin and rifabutin against four isolates of M. avium (for MICs for these strains, see Table 1). Although we did not observe any statistically significant activity of ethambutol when it was given alone to mice infected with these four isolates (data not shown), this agent was included as an example of dual therapy and because of previously reported synergy with the other agents.

In the case of strain 5-74, it was found (Fig. 3) that rifampin alone or in combination with ethambutol was able only to slow the infection and was not able to prevent dissemination to the bone marrow. In contrast, it was possible to achieve sterility in the liver, lungs, and bone

![FIG. 2. Growth of M. avium 5-74 in normal, TxCD4⁻, and beige mice. Mean values are shown (n = 4); the standard error of the mean did not exceed 0.35. ND, Not detected.](http://aac.asm.org/)

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Rifabutin (μg/ml)</th>
<th>Rifampin (μg/ml)</th>
<th>Ethambutol (μg/ml)</th>
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<td>6-12</td>
<td>&lt;0.15</td>
<td>5</td>
<td>2.5</td>
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<td>6-13</td>
<td>&lt;0.15</td>
<td>10</td>
<td>20</td>
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<tr>
<td>5-74</td>
<td>&lt;0.15</td>
<td>10</td>
<td>20</td>
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<tr>
<td>6-28</td>
<td>&lt;0.15</td>
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mice. Butol (P < 0.02, day 0.02, the mean group). Dual-therapy ethambutol combination and all marrow with rifabutin, while the activity of rifabutin in the spleen appeared to be enhanced by the inclusion of ethambutol (P < 0.02, day 120 spleens, rifabutin group versus dual-therapy group).

With strain 6-12 (Fig. 4), residual innate immunity in the TxCD4- mice appeared to be able to slowly clear the infection in most organs with the exception of the spleen, and all drugs tested tended to accelerate this activity (P < 0.02, day 120 spleens, all groups versus controls).

Strain 6-13 exhibited a higher level of virulence than the other strains tested, but it too was progressively cleared from the host by rifabutin therapy. In contrast, the rifampin-ethambutol combination had only limited activity (Fig. 5).

Finally, strain 6-28 proved to be the most difficult organism to contain, although in this case rifabutin and the rifampin-ethambutol combination had comparable bacteriostatic activities (P < 0.01) in the lungs of the infected mice (Fig. 6).

**FIG. 3.** Effect of chemotherapy on growth of *M. avium* 5-74 in TxCD4- mice. Mean values are shown (n = 4); the standard error of the mean did not exceed 0.33. ND, Not detected.

**FIG. 5.** Effect of chemotherapy on growth of *M. avium* 6-13 in TxCD4- mice. Mean values are shown (n = 4); the standard error of the mean did not exceed 0.29. ND, Not detected.

**DISCUSSION**

The results of this study show that thymectomized mice infused intravenously with monoclonal antibody specific for CD4+ T cells are more susceptible to *M. avium* infection than normal immunocompetent animals. The growths of the strain 5-74 infections in these mice were equivalent and sometimes in excess of growth observed in the established beige mouse model of this infection. Moreover, in keeping with the knowledge that innate immunity has a tendency to compensate in T-cell-deficient mice (an example being the chronic nature of listeriosis infection in immunocompromised mice; 2, 4), the TxCD4- mice used in these experiments did not show any evidence of increased susceptibility to common respiratory infections nor did they show any incidence on autopsy of spontaneous emergence of tumors. Thus, we suggest that the CD4-deficient mouse, which can easily be generated technically, may provide a useful alter-
native to the beige mouse in the modeling of *M. avium* infections in the immunodeficient host.

With this newly developed model, the results of this study confirm our previous contention (9) that the new antimycobacterial agent rifabutin has substantial activity in mouse models of infection, a finding in contrast to those of earlier studies that used the beige mouse. This discrepancy is almost certainly not a reflection of the particular mouse model used, however, but of the infection itself. In the earlier studies (6), a virulent strain, 101, of *M. avium* which exhibited only marginal susceptibility to a combination of clofazimine and rifabutin and to other regimens but which was substantially cleared from infected tissues by amikacin was used. Moreover, the rifabutin MIC for strain 101 is 2 μg/ml (6), whereas in our own experience, MICs for most *M. avium* isolates are in the 0.15- to 0.3-μg/ml range. However, we would argue that infections with organisms that display a large range of virulence (for mice) are likely to be found in AIDS patients and also that the activities of various individual drugs are likely to vary widely. Thus, we conclude that a particular regimen should be suggested for clinical consideration only if it proves to be effective against several different isolates in vitro.

In the present study, rifabutin showed evidence of substantial activity against three of four isolates studied. In addition, against one strain, supplementation of the regimen with ethambutol appeared to enhance the activity of rifabutin, suggesting synergy. In this regard, Heifets (7), Yajko and colleagues (14), Ozenne and colleagues (10), and Zimmer and colleagues (17) have made similar observations about rifampin-ethambutol combinations. Taken collectively, the results of the present and previous studies suggest that rifabutin may have significant activity against a majority of AIDS-associated isolates and should be considered as a part of multidrug regimens for treatment or prophylactic chemotherapy of AIDS.

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LITERATURE CITED


