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Contribution of Th1 and Th2 Cells to Protection and Pathology in Experimental Models of Granulomatous Lung Disease

Arun Wangoo,* Tim Sparer,* Ivor N. Brown,† Valerie A. Snewin,† Riny Janssen,† Jelle Thole,† H. Terence Cook,‡ Rory J. Shaw,* and Douglas B. Young 2†

Mice that had received adoptive transfer of DO11.10 TCR transgenic T cells polarized toward a Th1 or a Th2 phenotype were challenged with Ag-coated beads or with recombinant Mycobacterium tuberculosis expressing the OVA determinant. The resulting bead-induced pulmonary granulomas reflected the phenotype of the adoptively transferred T cells, with the Th2 cells promoting a fibrotic reaction. Mice receiving Th1 cells mounted an epitope-specific protective response to challenge with recombinant M. tuberculosis. Th2 recipients were characterized by enhanced weight loss and lung fibrosis during acute high-dose infection. The combination of TCR transgenic T cells and epitope-tagged mycobacteria provides a novel experimental model for investigation of the pathogenesis of tuberculosis. The Journal of Immunology, 2001, 166: 3432–3439.

Infection with Mycobacterium tuberculosis induces a complex immune response that includes multiple lymphocyte subsets and cytokines. Interactions between the different immune cells determine whether the infection results in progressive disease, clearance of the organism, or chronic persistence. The difficulty in distinguishing immune responses required for protection from those that contribute to disease pathology presents a central dilemma in the rational design of improved strategies for tuberculosis control.

There is compelling evidence that CD4+ T cells expressing the Th1 phenotype have an essential role in protection during mycobacterial infection. Mice with targeted deletion of these cells or the associated cytokines have markedly enhanced susceptibility to tuberculosis (1–4). In humans, tuberculosis is a common consequence of HIV-associated depletion of CD4+ T cells, and genetic polymorphisms affecting the Th1 response result in hypersusceptibility to mycobacterial infection (5, 6). In contrast, the significance of a Th2 response during mycobacterial infection is uncertain. In mice, the initial phase of M. tuberculosis infection is dominated by a Th1 response, with a Th2 response emerging after several weeks during the subsequent chronic phase of the disease (7, 8). The Th2 response may act to down-regulate T cells, reducing the extent of macrophage-mediated pathology, although perhaps at the same time contributing to persistence of the infection and the risk of reactivation (9). Alternatively, it has been proposed that the coexistence of Th1 and Th2 responses may exacerbate tissue damage by enhancing the pathological activity of TNF-α (10). The lack of any alteration in the course of M. tuberculosis infection in mice deficient in IL-4, a major Th2 cytokine, raises the further possibility that Th2 cytokines might represent a functionally irrelevant bystander response (11). Similar uncertainty applies to human tuberculosis, with contradictory reports describing the presence (12, 13) and absence of a Th2 component (14, 15). In two recent studies, the magnitude of the Th2 response in tuberculosis patients was found to correlate with the extent of disease progression, although it was noted that the relationship between cause and effect remained to be clarified (16, 17). The influence of an underlying Th2 response on the outcome of mycobacterial infection may be important in the context of age-related differences in susceptibility to tuberculosis and during coinfection with mycobacteria and intestinal parasites (18).

To investigate the contribution of the Th2 response to protection and pathology, we wished to establish an experimental model in which we could test the effect of deliberate addition of Th1 or Th2 cells during a mycobacterial infection. We elected to do this using the DO11.10 TCR transgenic model. The major T cell population in DO11.10 transgenic mice is CD4+ with an αβ TCR specific for peptide 323–339 from OVA presented in the context of I-A^d (19). Splenocytes from these mice have a Th0 profile but can be programmed by appropriate in vitro culture conditions to polarize toward a Th1 or Th2 phenotype (20, 21). DO11.10 T cells have been extensively characterized in a range of immunological systems, including adoptive transfer models (22–24). Our experimental model is based on adoptive transfer of DO11.10 T cells together with challenge with recombinant M. tuberculosis expressing the appropriate OVA epitope. In addition to the attraction of using a well-defined immunological system, this approach has the advantage of allowing us to distinguish between Ag-specific and non-specific effects, by carrying out parallel infections with a recombinant strain of M. tuberculosis expressing an irrelevant epitope.

We report on the contribution of Th1 and Th2 cells to protective and pathological responses in the lungs of mice challenged with OVA-coated beads or with OVA-expressing M. tuberculosis.

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Materials and Methods

Mice

Mice transgenic for the DO11.10 αβ TCR on a BALB/c background, were obtained from F. Powrie at the Nuffield Institute (Oxford, U.K.) and were originally developed by D. Loh at DNAx (Palo Alto, CA) (19). These mice were bred and maintained at Imperial College facilities at St. Mary’s campus and were checked for transgenes by staining splenocytes with the antitumor monoclonal antibody KJL-26 (25).

For adoptive transfer recipients, female nontransgenic BALB/c mice raised under specific pathogen-free conditions were purchased from Harlan OLAC (Bicester, U.K.). The mice were maintained for at least 1 wk and used for experiments at 7–8 wk of age.

Th1/Th2 culture protocol

Splenocytes from DO11.10 mice were isolated on a Ficoll-Paque gradient (Pharmacia, Uppsala, Sweden) and cultured in 24-well culture plates at 1 × 10^6 cells/ml in RPMI 1640 medium containing 5 mM HEPES buffer (Life Technologies, Rentfrewshire, U.K.), 10% FCS (ICN/Flow Laboratories, High Wycombe, U.K.), 100 U/ml penicillin and 100 µg/ml streptomycin (Life Technologies), 2 mM glutamine (Life Technologies), and 0.05 µM 2-ME (Sigma, Poole, U.K.). Cells were cultured with OVA peptide (1 µM) together with 10 U/ml IL-12 (R&D Systems, Abingdon, U.K.) and 10 µg/ml anti-IL-4 (PharMingen, San Diego, CA) to generate the Th1 phenotype. For Th2 cells, IL-12 and anti-IL-4 were replaced by 200 U/ml IL-4 (Genzyme, Cambridge, MA). Cells were cultured in presence of OVA alone for the Th0 phenotype. The OVA peptide, corresponding to amino acids 323–339 of chicken OVA, was synthesized by Dr J. W. Driffler (Biocenter, Basel, Switzerland) and was checked for purity by amino acid analysis, HPLC, and mass spectrometry. Plasmids were introduced into Escherichia coli on an electrocompetent strain, and hygromycin-resistant transformants characterized by electrophoresis on an agarose gel. Oligonucleotides encoding amino acids 323–339 of chicken OVA, was synthesized by Dr J. W. Driffler (Biocenter, Basel, Switzerland) and was checked for purity by amino acid analysis, HPLC, and mass spectrometry. Plasmids were introduced into Escherichia coli on an electrocompetent strain, and hygromycin-resistant transformants characterized by electrophoresis on an agarose gel.

Preparation of OVA-coated beads

Cyanogen bromide-activated Sepharose 4B beads (Sigma) were swollen and coupled with OVA according to the manufacturer’s protocol. Briefly, 1 g of Sepharose beads were swollen in 1 mM HCl, then washed and suspended in 5 ml of coupling buffer (0.1 M NaHCO₃, 0.5 M NaCl) containing 10 µM OVA. The mixture was rotated end-over-end on a rotary mixer overnight at 4°C. Excess peptide was removed by washing with 5 gel volumes of coupling buffer, and remaining reactive sites blocked with 0.1 M Tris HCl (pH 8) for 2 h. Beads were stored at 4°C in sterile PBS containing 0.05% NaN₃. Beads were washed three times in sterile PBS and counted microscopically before injection.

OVA-coated or uncoated control beads (6000 per mouse) were introduced into normal BALB/c mice by i.v. injection as described by Chensue et al. (26). After 24 h, DO11.10 transgenic T cells were administered by a second i.v. injection. mice were sacrificed on days 3, 7, and 21 postinjection. Pathology was monitored in the lungs, liver, and spleen. In some experiments, single cell suspensions of splenocytes were checked for expression of Th1 and Th2 phenotypes by culturing in presence of OVA for 48 h.

Recombinant M. tuberculosis and mycobacterial infection

M. tuberculosis H37Rv was grown with shaking at 37°C in Middlebrook 7H9 liquid medium supplemented with albumin, dextrose and catalase (ADC) as recommended by the manufacturer (Difco). When appropriate, hygromycin B (Boehringer Mannheim, Postfach, Germany) was added at 50 µg/ml. To generate recombinant constructs, oligonucleotides encoding amino acids 323–339 of chicken OVA and 120–138 of influenza virus hemagglutinin were cloned within the M. tuberculosis gene cluster on an Escherichia coli mycobacteria shuttle plasmid as described previously (27). Plasmids were introduced into M. tuberculosis by electroporation, and hygromycin-resistant transformants characterized by electro

phoretic analysis of the modified superoxide dismutase protein in sonicated preparation under denaturing and non-denaturing conditions (27). Confirmed recombinant clones were stored at −70°C as stock cultures containing 10^8 CFU/ml.

For infection experiments, thawed aliquots were mixed with DO11.10 cells and injected i.v. into mice at a challenge dose of ~2 × 10^6 CFU per mouse. An aliquot of the suspension was plated on 7H11 agar to determine the precise infecting dose. Groups of three mice representing each of the T cell/challenge conditions were sacrificed on days 7 and 21 postinfection. Mice were weighed, and lungs, liver, and spleen were removed for analysis.

To measure bacterial load, samples of infected tissue were weighed and homogenized in 3 ml saline in a stomacher. Homogenates were then plated on Middlebrook 7H11 agar plates in serial 10-fold dilutions in duplicate and incubated at 37°C for 2 wk. In some experiments, parallel sets of plates were prepared with and without hygromycin. Colonies were counted and the number of CFUs per organ was calculated.

Preparation of histological specimens

Organs were weighed before a small portion of each tissue was removed and fixed by immersion in 10% formal saline solution. Samples were embedded in paraffin wax, cut into sections, and mounted onto slides. Sections were then stained with hematoxylin to assess the extent of granuloma formation. In the Sepharose bead model, the size of the granulomas in lungs was measured by microscopy (×400 magnification) using image analyzer software. The inflammatory mass was determined by measurement of at least 20 granuloma for each condition. For M. tuberculosis infection, the lung infiltrates and granuloma formation were graded on a semiquantitative scale. Sections were coded and examined (by H.T.C.) without knowledge of experimental code.

Hydroxyproline assay

Hydroxyproline content was determined by the methods described by Stegeman and Stalder (28). Briefly, a portion of lungs was hydrolyzed in 6 N HCl at 100°C overnight. The sample was mixed with chloramine T solution and incubated for 20 min at room temperature. After incubation, aldehyde/perchloric acid solution was added and incubated at 60°C. After 15 min, the samples were cooled under tap water and absorbance was measured at 550 nm. The hydroxyproline content was expressed for whole lungs.

Isolation and RT-PCR amplification of mRNA

Small pieces of lung tissues were snap frozen in liquid nitrogen and stored at −70°C. Total RNA from the frozen tissue was isolated by homogenizing the organs in 0.5 ml of guanidine isothiocyanate. RNA was extracted by modification of acid-GTC phenol chloroform method as described previously (29). First-strand cDNA synthesis from 1 μg total RNA was conducted as described by Wang and co-workers (30). The gene-specific primers were as follows: β-actin, 5′-GATGTTGCTCAGCTCCTCA-3′ (sense), 5′-AACTGCTACA CAGTGATCT-3′ (antisense); IL-13, 5′-GCCAGCCACGTATCAG-3′ (sense), 5′-ATGGATGAGCGATATCGCT-3′ (antisense); IL-13, and collagen III, 5′-CTGATGAGCTGATCACTGGGC-3′ (antisense); collagen III, 5′-TGATTACACAGGCTGCCTCA-3′ (antisense), 5′-GCTAGATGAGCTGACACAG-3′ (antisense). The predicted sizes of β-actin, IL-13, and collagen III DNA products were 370, 398, 161, and 257 bp, respectively.

To permit the same number of cycles in the PCR to be used for measurement of each of the cDNAs, and, thus, for the assays to be performed concurrently, cDNA was diluted 1:10 for IFN-γ, IL-13, and collagen III, and 1:50 for β-actin. A 5-fold correction factor was subsequently used to calculate β-actin ratios. The PCR amplification mixture consisted of 10× PCR buffer, 1.25 mM MgCl₂, 1 U of Taq polymerase (Promega, Southampton, U.K.), 200 µM deoxyribonucleotides (dATP, dGTP, dCTP, and dTTP), 0.3 µM of each primer, and appropriate dilution of cDNA, made up to 50 µl with sterile distilled water. Amplification was conducted for 33 cycles in a DNA thermal cycler (Perkin-Elmer, Beaconsfield, U.K.) as follows: 94°C for 1 min, 55°C for 2 min, and 72°C for 2 min. PCR products (15 µl) were separated by electrophoresis on a 2% agarose gel containing ethidium bromide. For visualization of the bands, the gel was photographed under UV light.

Statistical analysis

Data were compared using the Mann-Whitney U test, and a p value of <0.05 was taken to be statistically significant. Error bars represent one SE from the mean.
Results

Establishment of Th1 and Th2 cells

Splenocytes from DO11.10 transgenic mice were cultured with OVA for 1 wk in different cytokine environments to polarize them toward the Th1 or Th2 phenotype. Cells were then washed, restimulated with peptide alone for 48 h, and supernatants analyzed for IFN-γ and IL-5. Table I records a representative experiment. Cells cultured in the absence of added cytokine had a Th0 profile, expressing minimal amounts of IFN-γ or IL-5. Culture in the presence of IL-12 and anti-IL-4 Ab generated cells with a Th1 profile, characterized by a 70-fold increase in IFN-γ production. Conversely, cells cultured in the presence of IL-4 expressed 20-fold higher levels of IL-5, characteristic of a Th2 response.

Stability of cytokine profile on adoptive transfer

To test the stability of the Th1/Th2 polarization, splenocytes from in vitro cultures were adoptively transferred into normal BALB/c mice that had been injected with OVA-coated Sepharose beads 24 h earlier. One week later, spleens were removed from the mice and analyzed for expression of IFN-γ and IL-5 in response to restimulation with OVA. The polarized T cells were found to have retained their phenotype in vivo (Fig. 1). Spleen cells from mice receiving Th1 cells secreted IFN-γ when restimulated with OVA in vitro; spleen cells from mice receiving Th2 cells produced a higher amount of IL-5.

Influence of Th1 and Th2 cells on formation of bead-induced granulomas in the lung

We next wished to determine whether adoptively transferred DO11.10 T cells would influence immune responses in the lungs of recipient animals. We initially explored a simple Sepharose bead model in which OVA-coated beads were injected i.v. into BALB/c mice and embolized in the lung. Th1, Th2, or a mixture of both Th1 and Th2 cells was similarly injected via the tail vein 24 h later. Mice were sacrificed after 7 days and the lungs removed for examination. On histological analysis, it was evident that the granulomas that formed around the beads in mice injected with Th1 or Th2 cells were considerably larger than those in mice injected with beads alone (Fig. 2, A–C). This was confirmed by quantitation of the inflammatory mass using an image analyzer (Fig. 2D). The inflammatory mass was similar in mice receiving Th1 cells, Th2 cells, or a mixture of the two phenotypes. However, a difference was evident from measurement of lung hydroxyproline content as a marker of fibrosis. Although all of the mice receiving DO11.10 cells showed an increase in hydroxyproline content over controls receiving beads alone, hydroxyproline content was higher with Th2 cells, or Th2 together with Th1 cells, than with Th1 alone (Fig. 3).

Responses in the lung were further explored using RT-PCR to measure mRNA encoding cytokines and collagen III. Using mRNA for β-actin as a positive control for the RT-PCR assay, samples were scored as positive or negative based on the presence or absence of a visible band on gels stained with ethidium bromide. The results are summarized in Table II. Lungs from mice receiving Th1 cells were positive for IFN-γ, a Th1 marker, and predominantly negative for the Th2 cytokine IL-13. The reverse was true in recipients of Th2 cells. Lungs from mice receiving a mixture of both cell types were predominantly positive for both cytokines. The intensity of RT-PCR bands for IFN-γ and IL-13 in lungs from mice receiving the Th1/Th2 combination was markedly lower than in mice receiving the individual cell types (Fig. 4). This could be the result of reciprocal competition between the two cell types (31) or simply due to transfer of a lower total number of each cell type in the mixed population. Expression of collagen III mRNA, an indicator of fibrosis, was seen exclusively in lungs from the mice

Table I. Cytokine production by spleen cells from TCR-transgenic mice

<table>
<thead>
<tr>
<th>Culture Conditions (7 days) and Restimulation (2 days)</th>
<th>OVA Alone</th>
<th>OVA + IL-12</th>
<th>OVA + IL-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>OVA (0.1 μM)</td>
<td>IFN-γ</td>
<td>1.39 ± 0.39</td>
<td>62.89 ± 16.06</td>
</tr>
<tr>
<td></td>
<td>IL-5</td>
<td>0.30 ± 0.02</td>
<td>0.34 ± 0.08</td>
</tr>
<tr>
<td>OVA (0.3 μM)</td>
<td>IFN-γ</td>
<td>3.99 ± 1.19</td>
<td>293.17 ± 28.11</td>
</tr>
<tr>
<td></td>
<td>IL-5</td>
<td>0.33 ± 0.08</td>
<td>0.41 ± 0.25</td>
</tr>
<tr>
<td>OVA (1.0 μM)</td>
<td>IFN-γ</td>
<td>10.45 ± 2.46</td>
<td>698.24 ± 33.10</td>
</tr>
<tr>
<td></td>
<td>IL-5</td>
<td>0.30 ± 0.02</td>
<td>1.52 ± 0.74</td>
</tr>
</tbody>
</table>

* Cytokine production is expressed as nanograms per milliliter in supernatants from cultures each containing 1 × 10⁶ splenocytes. SE are shown from a representative experiment. The same pattern was observed in five independent cultures.
that received Th2 cells, either alone or in combination with Th1 cells. Again, the RT-PCR product was more prominent in the Th2 only group.

Influence of Th1 and Th2 cells on infection with M. tuberculosis

Having shown that the DO11.10 T cells retained their phenotype after adoptive transfer and were able to influence responses in the lung, we next investigated the course of M. tuberculosis infection in mice receiving Th1 or Th2 cells. For this, we first constructed recombinant strains of M. tuberculosis expressing either the OVA peptide or a control peptide from influenza virus hemagglutinin (HA). In each case, an oligonucleotide encoding the appropriate amino acid sequence was inserted within a permissive loop of the M. tuberculosis superoxide dismutase protein and the recombinant construct reintroduced into M. tuberculosis H37Rv using a shuttle vector (27). BALB/c mice were injected i.v. with Th0, Th1-, or Th2-polarized DO11.10 splenocytes together with M. tuberculosis recombinants expressing either OVA or HA. Mice were sacrificed on days 7 and 21, and the lungs were removed for examination.

These experiments demonstrate that the adoptively transferred cells were able to influence immune responses in the lungs of recipient animals and that the Th2 cells promoted a fibrotic reaction characterized by collagen III expression and formation of hydroxyproline.

FIGURE 2. Characterization of bead-induced pulmonary granulomas. Granulomatous inflammation surrounding OVA-coated beads in the lungs of mice receiving adoptive transfer of Th1 cells (A), Th2 cells (B), or no cells (C) was examined on day 7 postinjection (×400 magnification). Quantitation of the inflammatory mass by image analyzer (D) showed a similar increase in response to each of the T cell subsets compared with controls receiving beads alone.

Table II. RT-PCR detection of mRNA in lungs from mice injected with OVA-coated beads and adoptively transferred T cells

<table>
<thead>
<tr>
<th></th>
<th>β-actin</th>
<th>IFN-γ</th>
<th>IL-13</th>
<th>Collagen III</th>
</tr>
</thead>
<tbody>
<tr>
<td>OVA beads + Th1 cells</td>
<td>11/11</td>
<td>11/11</td>
<td>2/5</td>
<td>1/11</td>
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<tr>
<td>OVA beads + Th2 cells</td>
<td>11/11</td>
<td>0/11</td>
<td>5/5</td>
<td>10/11</td>
</tr>
<tr>
<td>OVA beads + Th1 and Th2</td>
<td>5/5</td>
<td>4/5</td>
<td>5/5</td>
<td>3/5</td>
</tr>
<tr>
<td>OVA beads alone</td>
<td>5/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Beads alone</td>
<td>5/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
</tbody>
</table>

*Results are expressed as the number of mice with a positive band on the gel/total mice tested.

3 Abbreviation used in this paper: HA, hemagglutinin.
A similar bacterial load was observed in lungs removed from all six groups of mice on day 7 after infection. However, a significant difference was observed on day 21 (Fig. 5). The bacterial load in the lungs of the mice infected with M. tuberculosis OVA in the presence of Th2 cells was 10-fold higher than in the Th1 mice. In mice receiving Th0 cells, the bacterial load was intermediate; closer to that in the Th2 recipients but significantly different from either of the other two groups \((p = 0.02)\). In contrast, no difference was observed between the three groups challenged with an equivalent dose \((1-2 \times 10^6\) CFU) of M. tuberculosis HA. The same trend was observed after challenge with a higher dose of M. tuberculosis. Again, there was no difference in bacterial load at day 7, but at day 21, lung counts from Th2 mice \((8.5 \pm 2.5 \times 10^7\) CFU) were significantly greater than in Th1 mice \((1.7 \pm 0.7 \times 10^7\) CFU), although only slightly higher than the Th0 group \((7.4 \pm 1.8 \times 10^7\) CFU). These results demonstrate that transfer of the Th1 cells enhanced the ability of the mice to control the mycobacterial infection. The presence of Th2 cells had a smaller reverse effect. Neither cell phenotype influenced infection with the HA control strain lacking the specific antigenic target.

Further analysis was conducted to determine whether T cell transfer had altered pathological manifestations of the infection. During the lower dose infection, the weight of the mice in each of the groups remained approximately constant. Most of the mice lost weight during the course of the high-dose infection, but weight loss was significantly greater in the group receiving Th2 cells and challenged with M. tuberculosis OVA (Fig. 6A). Conversely, the lung weight was highest in the Th2 group. This was most clearly seen in the lower dose challenge, during which overall body weight remained comparable between the groups (Fig. 6B). Hydroxyproline content was significantly higher in Th2 compared with Th1 lungs \((p = 0.02)\) (Fig. 6C). No pathological differences were observed between the three groups challenged with M. tuberculosis HA.

Sections of the lungs from mice infected with M. tuberculosis for 21 days were examined by a histopathologist unaware of the experimental code and scored for cellular infiltration and granuloma formation. There was a clear increase in the amount of inflammation in Th2 recipients compared with Th1 recipients. This is illustrated by a low-power view of representative sections in Fig. 7. Lungs from the Th0 group were scored as showing an intermediate level of infiltration. More detailed examination revealed filling of the alveoli by an infiltrate of mononuclear inflammatory cells and neutrophils. Although the infiltrate was more extensive in the Th2 mice, there was no evidence of any significant qualitative differences in the nature of the lesions among the different groups. No eosinophilia was observed in any of the lung sections. Histological examination of liver sections from infected mice similarly revealed quantitative but not qualitative differences in granuloma formation.

**FIGURE 4.** RT-PCR gel profiles for mRNA in representative samples from mice injected with OVA-coated beads and adoptively transferred T cells.

**FIGURE 5.** Effect of Th1 and Th2 cells on growth of M. tuberculosis. CFUs were measured in homogenates of lungs removed from mice 7 and 21 days after infection with recombinant M. tuberculosis together with Th1, Th2, or Th0 cells. Growth of OVA-expressing M. tuberculosis was markedly reduced in lungs of mice receiving OVA-specific Th1 cells. There was a slight increase in CFUs of mice receiving Th2 cells in comparison to the Th0 group. Cell transfer had no effect on growth of control M. tuberculosis expressing the HA epitope. Results are shown as mean ± SE. Statistically significant differences among groups \((p < 0.05)\) are indicated by asterisks.

**Discussion**

Intravenous injection of Ag-coated beads has been used extensively as a model to study granuloma formation. When introduced into appropriately immunized mice, beads coated with mycobacterial Ags or with schistosomal egg Ags have been shown to give rise to type-1 or type-2 granulomas, respectively (26). Type-1 granulomas are characterized by expression of Th1 cytokines, TNF-\(\alpha\), and the chemokine RANTES (26, 32). Type-2 granulomas resemble those induced in response to intact eggs from Schistosoma mansoni (33) and are characterized by Th2 cytokines, recruitment of eosinophils, and the deposition of collagen (26). The granulomas formed around OVA-coated beads after adoptive transfer of Th1- or Th2-polarized DO11.10 cells in the present study conform to the type-1/type-2 paradigm, with raised levels of hydroxyproline and collagen III expression in granulomas formed in the Th2 recipients. Fibrosis in granulomatous lesions is regulated by a complex network of fibroblasts and lymphocytes. Collagen biosynthesis is stimulated by Th2 cytokines, such as IL-4 and IL-13, and reciprocally inhibited by the Th1 cytokines, IFN-\(\gamma\) and IL-12 (34, 35). The observations with the adoptive transfer system are consistent with the hypothesis that fibrosis is stimulated by the direct action of Th2 cytokines, rather than as an indirect

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**FIGURE 5.** Effect of Th1 and Th2 cells on growth of M. tuberculosis. CFUs were measured in homogenates of lungs removed from mice 7 and 21 days after infection with recombinant M. tuberculosis together with Th1, Th2, or Th0 cells. Growth of OVA-expressing M. tuberculosis was markedly reduced in lungs of mice receiving OVA-specific Th1 cells. There was a slight increase in CFUs of mice receiving Th2 cells in comparison to the Th0 group. Cell transfer had no effect on growth of control M. tuberculosis expressing the HA epitope. Results are shown as mean ± SE. Statistically significant differences among groups \((p < 0.05)\) are indicated by asterisks.

**Discussion**

Intravenous injection of Ag-coated beads has been used extensively as a model to study granuloma formation. When introduced into appropriately immunized mice, beads coated with mycobacterial Ags or with schistosomal egg Ags have been shown to give rise to type-1 or type-2 granulomas, respectively (26). Type-1 granulomas are characterized by expression of Th1 cytokines, TNF-\(\alpha\), and the chemokine RANTES (26, 32). Type-2 granulomas resemble those induced in response to intact eggs from Schistosoma mansoni (33) and are characterized by Th2 cytokines, recruitment of eosinophils, and the deposition of collagen (26). The granulomas formed around OVA-coated beads after adoptive transfer of Th1- or Th2-polarized DO11.10 cells in the present study conform to the type-1/type-2 paradigm, with raised levels of hydroxyproline and collagen III expression in granulomas formed in the Th2 recipients. Fibrosis in granulomatous lesions is regulated by a complex network of fibroblasts and lymphocytes. Collagen biosynthesis is stimulated by Th2 cytokines, such as IL-4 and IL-13, and reciprocally inhibited by the Th1 cytokines, IFN-\(\gamma\) and IL-12 (34, 35). The observations with the adoptive transfer system are consistent with the hypothesis that fibrosis is stimulated by the direct action of Th2 cytokines, rather than as an indirect
consequence of their effect on Th1 responses. Adoptive transfer of in vitro polarized T cells provides an additional approach that complements existing gene knockout and cytokine treatment models for analysis of the dynamics of granuloma formation (36–40).

Infection of the DO11.10 recipient mice with virulent *M. tuberculosis* presents a more complex immunological challenge. We elected to use a relatively high-dose i.v. challenge for these initial experiments with the aim of ensuring rapid evolution of the infection in the context of a high circulating level of the adoptively transferred cells and also of maximizing the opportunity of identifying alterations in pathology. We used intact BALB/c mice in preference to T cell-depleted animals as recipients because we wished to analyze the effect of adding defined T cell phenotypes on top of the complex natural antimycobacterial response. A striking finding from the study is that adoptive transfer of Th1 cells resulted in a 10-fold reduction in mycobacterial multiplication during the first three weeks of the acute infection. This effect is of a similar magnitude to that obtained by BCG vaccination or by adoptive transfer of splenocytes from mice exposed to *M. tuberculosis* infection (41). Splenocyte cultures used for adoptive transfer contain cell types in addition to the OVA-specific DO11.10 T cells. However, the absence of any influence on infection with *M. tuberculosis* expressing the irrelevant HA epitope indicates that the effects observed during *M. tuberculosis* OVA challenge are mediated by the epitope-specific TCR-transgenic cells. A similar level of protection in the acute i.v. challenge model has previously been described after adoptive transfer of a clonal CD8+ T cell population expressing IFN-γ (42). Some reservations must be expressed concerning the extent to which these results can be extrapolated to a more physiological low-dose aerosol challenge (43), but the fact that this level of protection can be generated by a T cell population directed toward a single antigenic determinant is of interest in the context of attempts to develop subunit vaccines against tuberculosis. Despite the large number of Ags presented by a mycobacterial pathogen, an appropriate T cell response to one epitope is sufficient to significantly alter the course of infection. Further manipulation of the DO11.10 model, for example, by expression of the OVA determinant on protein carriers expressed in different subcellular locations (44), may generate data relevant to rational vaccine design.

The OVA epitope in the challenge strain is carried on a plasmid vector routinely maintained in the mycobacteria by antibiotic selection (27). The absence of antibiotic selection in vivo, in combination with the possible immunological counterselection by Th1 cells, encouraged us to screen for plasmid loss during infection. However, plating of lung homogenates in the presence and absence

![Figure 6](http://www.jimmunol.org/)

**Figure 6.** Effect of Th1 and Th2 on pathology during *M. tuberculosis* infection. Lungs from mice infected 21 days previously with recombinant *M. tuberculosis* together with Th1, Th2, or Th0 cells were examined for markers associated with disease pathology. Recipients of Th2 cells were characterized by loss of total body weight, together with increased lung weight and hydroxyproline content. This effect was specific to infection with OVA-expressing *M. tuberculosis*. Results are shown as mean ± SE, with statistically significant differences among groups (p < 0.05) indicated by asterisks.

A. Total body weight is shown for each of the groups on days 7 and 21 after a high-dose challenge with *M. tuberculosis*. In comparison to the other groups, weight loss by day 21 was significantly greater for the Th2 recipients challenged with OVA-expressing *M. tuberculosis*. (Body weight remained constant for all groups during a lower dose challenge.) B. Lung weight expressed as a percentage of total body weight is shown for each of the groups on day 21. Results are shown after a lower dose challenge with *M. tuberculosis*, during which total body weight remained constant for all groups. Lung weight was highest in the Th2 recipients infected with *M. tuberculosis* OVA. C. Hydroxyproline content in lungs is shown for each of the groups (lower dose challenge). After infection with *M. tuberculosis* OVA, hydroxyproline content was significantly lower in the Th1 group compared with the Th2 group.
of hygromycin provided no evidence of measurable plasmid loss in any of the groups at the 21-day time point. Additional experiments monitoring plasmid stability over a longer time course may provide a means of measuring Th1 immune pressure on mycobacterial growth.

The effect of Th2 cell transfer on mycobacterial growth was less marked. The modest increase in bacterial load in Th2 compared with Th0 recipients is consistent with a possible antagonistic effect of Th2 cytokines on development of the natural Th1 response to the infection (31). The weight loss in Th2 mice challenged with the high dose of M. tuberculosis OVA and the increased lung weight suggest some exacerbation in the pathology of the infection. This could be attributed to a direct contribution of Th2 cells to pathology or may simply reflect more advanced disease due to the enhanced mycobacterial growth in these mice. Although lesions were more extensive in the Th2 lungs, there was no evidence of any qualitative change indicative of Th2 modulation of the inflammatory process. Consistent with previous observations in a BCG-induced granuloma model (45), hydroxyproline content was significantly reduced in mice exposed to an augmented Th1 response. Although lesions were more extensive in the Th2 lungs, there was no evidence of any qualitative change indicative of Th2 modulation of the inflammatory process. Consistent with previous observations in a BCG-induced granuloma model (45), hydroxyproline content was significantly reduced in mice exposed to an augmented Th1 response. Hydroxyproline levels were similar in the Th0 and Th2 recipients.

It will be of considerable interest to extend the DO11.10 model over a more prolonged course of infection. Transfer of Th2 cells subsequent to the initial Th1-dominated phase of the response may have a more marked impact on disease progression, for example, providing an opportunity to examine the contribution of profibrotic events to mycobacterial persistence. The ability to track adaptively transferred T cells using clonotypic Abs may also assist in investigation of the anatomical location of mycobacteria in low-dose persistence models.

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