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T Cells Require Tumor Necrosis Factor- α to Provide Protective Immunity in Mice Infected with *Histoplasma capsulatum*

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Abstract

We examined whether neutralization of tumor necrosis factor (TNF)- α after intranasal exposure of mice to *Histoplasma capsulatum* was necessary for control of primary or secondary infection. All mice given monoclonal antibody to TNF- α on the day of infection or on day 3 after infection died. When antibody was administered on day 5 after infection, 60% of mice with primary infection died, whereas none with secondary infection did. Antibody treatment on day 7 after infection produced a transiently higher fungal burden. Because optimal clearance required TNF- α after the onset of infection, we hypothesized that it may regulate T cell function. Lung CD3⁺ cells were the dominant population of TNF- α -producing cells (~40%-70%). Neutralization of this cytokine decreased the number of memory T cells but not the number of activated, proliferating, or interferon- γ -producing cells. T cells from infected, TNF- α -neutralized mice failed to protect T cell-deficient mice. The absence of TNF- α induces a defect in T cell-mediated protection.

Resolution of infection with *Histoplasma capsulatum* requires interaction between cellular and molecular effectors. T cells, dendritic cells, and macrophages are the dominant cellular determinants [1-9]. Tumor necrosis factor (TNF)- α , interferon (IFN)- γ , granulocyte-macrophage colony-stimulating factor, and interleukin (IL)-12 contribute to elimination of the fungus [10-17]. By contrast, IL-4 and IL-10 appear to dampen the host response to infection [10,11,18-20]. TNF- α is a central mediator of host defenses. It is necessary for protective immunity in both primary and secondary infection in mice [1,11,16,20]. The introduction of TNF- α inhibitors into the clinical arena has been accompanied by increasing reports of infections with *H. capsulatum* [21,22] as well as *Mycobacterium tuberculosis* [23,24]. These reports document the necessity for endogenous TNF- α in humans.

The mechanisms by which TNF- α contributes to the protective immune response to *H. capsulatum* infection are poorly understood. In primary infection, the absence of this cytokine is associated with impaired production of nitric oxide, which is essential for host control of infection [11,20]. In secondary infection, the absence of TNF- α is associated with increases in levels of IL-4 and IL-10, which causes exacerbation of infection [11]. Because TNF- α modulates several properties of T cells, including expansion and antigen responsiveness [25, 26], we explored the influence of this cytokine on T cell function.

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MATERIALS AND METHODS

Mice. Male C57BL/6 and T cell receptor (TCR) $\alpha\beta^{-/-}$ mice were purchased from Jackson Laboratories. All animal experiments were done in accordance with the Animal Welfare Act guidelines of the National Institutes of Health.

Preparation of *H. capsulatum* and infection of mice. *H. capsulatum* yeast (strain G217B) was prepared as described elsewhere [1]. To produce primary infection, mice were infected intranasally with 2×10^6 *H. capsulatum* yeasts in 30 μ L of Hanks' balanced salt solution (HBSS). For secondary histoplasmosis, mice were inoculated with 1×10^4 yeasts intranasally in 30 μ L of HBSS. Six to eight weeks later, mice were rechallenged intranasally with 2×10^6 yeasts.

Organ culture for *H. capsulatum*. *H. capsulatum* was recovered from cultures as described elsewhere [1]. Fungal burden was expressed as the mean $\pm$ SE number of colony-forming units per whole organ. The limit of detection was 1×10^2 cfu.

Treatment of mice with neutralizing monoclonal antibody (MAb) to TNF- α . Rat anti-mouse TNF- α (from cell line XT-22.1) was purchased from the National Cell Culture Center and purified. The cell line was obtained from J. Abrams (DNAX). Mice were injected intraperitoneally with 1 mg of MAb to TNF- α . This amount of MAb suppresses endogenous TNF- α for 7 days in *H. capsulatum*-infected mice (data not shown). Control mice received an equal amount of rat IgG.

Isolation of lung leukocytes and splenocytes. Lung leukocytes were isolated as described elsewhere [27]. Splenocytes were obtained by teasing apart spleens between the frosted ends of 2 glass slides. Cells were washed 3 times with HBSS before being examined.

Flow cytometry. To determine the phenotype of TNF- α -producing cells, lung leukocytes and splenocytes were adjusted to a concentration of 2×10^6 cells/200 μ L of staining buffer (consisting of PBS [pH 7.4], 2% bovine serum albumin, and 0.02% sodium azide [PBSA]) and were incubated with 0.5 μ g of allophycocyanin-labeled MAbs (BD Biosciences) to one of the following: CD3 ϵ (clone 145-2C11), Ly-6G (Gr-1; clone RB6-8C5), CD11c (clone HL3), TCR β chain (clone H57-597), or F4/80 (clone AI-C3-1; Caltag Laboratories). To determine the expression of intracellular TNF- α , surface-stained cells were washed several times in Perm/Wash buffer (BD Biosciences), fixed and permeabilized with Cytofix/Cytoperm (BD Biosciences), and stained with phycoerythrin-conjugated MAb to TNF- α (clone XT-22.1; 2.5 μ g/ 1×10^6 cells). The cells were fixed in 2% paraformaldehyde. To determine intracellular IFN- γ expression, cells were stained with allophycocyanin-conjugated MAb to CD3 ϵ , followed by permeabilization of cells and staining with phycoerythrin-conjugated anti-IFN- γ .

The proportion of cells bearing a memory phenotype was determined by incubating lung leukocytes with MAbs (BD Biosciences) to CD3 ϵ -peridinin chlorophyll protein, CD44-phycoerythrin (clone Pgp-1, Ly-24), and CD62L-allophycocyanin (L-selectin, clone MEL-14). The samples were washed and fixed in 2% paraformaldehyde. To identify CD3 $^+$ CD69 $^+$ cells from the lungs, cells were stained with allophycocyanin-labeled MAb to CD3 ϵ and phycoerythrin-labeled CD69 (clone H1.2F3; BD Biosciences).

Bromodeoxyuridine staining of T cells. Groups of mice were injected intraperitoneally daily with 100 μ g of bromodeoxyuridine (BD Biosciences) in HBSS. Aliquots of 1×10^6 lung leukocytes were frozen in 1 mL of 60% RPMI 1640, 30% fetal bovine serum, and 10% dimethylsulfoxide. Cells were thawed, washed twice with PBSA, and incubated for 15 min on ice with MAb to CD3-allophycocyanin. The cells were washed with staining buffer, resuspended in 100 μ L of Cytofix/Cytoperm, and incubated at room temperature for 15 min.

Cells were washed with Perm/Wash buffer, resuspended in 100 μ L of Cytoperm Plus buffer, incubated on ice for 10 min, and washed with Perm/Wash buffer. Cells were incubated with Cytofix/Cytoperm for 5 min at room temperature. Cells were treated with 30 μ g of DNase for 1 h at 37 °C, washed, and resuspended in Perm/Wash buffer containing fluorescein isothiocyanate-labeled antibody to bromodeoxyuridine. After 20 min, cells were washed, resuspended in buffer, and analyzed.

Purification of T cells. Lung leukocytes from mice infected for 1 week with 2×10^6 *H. capsulatum* and given either 1 mg of rat IgG or 1 mg of MAb to TNF- α were prepared as described elsewhere [27]. Cells were adjusted to 2×10^7 cells/mL, layered over 2-5 mL of Lympholyte-M (Cedarlane Laboratories), and centrifuged at 1500 g for 20 min. Cells were washed in PBS supplemented with 0.5% bovine serum albumin and 2 mmol/L EDTA. Cells were adjusted to 1×10^7 cells/90 μ L in PBS. Four hundred microliters of CD90 microbeads (Miltenyi Biotec) was added to cells, for a final concentration of 1×10^7 cells/10 μ L. After a 15-min incubation at 6-12 °C, cells were washed and resuspended in PBS. Cells were percolated through magnetic-activated cell sorting columns (Miltenyi). Columns were washed 3 times with PBS. Columns were removed from the magnetic field, and cells were flushed with PBS. The positive fraction was washed twice with HBSS. The purity of CD3⁺ cells exceeded 95%, as was shown by flow cytometry. No yeasts were observed or recovered (limit of detection, 10 cfu) in the T cell fraction.

Adoptive transfer. Two days before intranasal infection with 2×10^6 yeasts, TCR $\alpha\beta^{-/-}$ mice were injected intraperitoneally with 2.5×10^6 T cells from 1-week-infected mice given rat IgG or MAb to TNF- α . Cells were suspended in HBSS and injected in a 0.5-mL volume. As a control, a group of mice received only 0.5 mL of HBSS.

Statistics. Analysis of variance (ANOVA) was used to compare groups. The log-rank test was used to assess differences in survival.

RESULTS

Requirement that TNF- α production be sustained to mediate protective immunity. Naive mice were infected with *H. capsulatum* intranasally and given MAb to TNF- α at the time of infection or on day 3 or 5 after infection. On day 7, mice were killed, and fungal burden was assessed in lungs and spleens. Mice treated with MAb on days 0 and 3 manifested a striking increase in fungal burden in both lungs and spleens, compared with burdens in infected control mice ($P < .01$, ANOVA). If treatment was begun on day 5 after infection, the increase in fungal burden was more modest yet was still significant ($P < .05$) (figure 1A and 1B). Because treatment as late as day 5 after infection induced a marked alteration in the number of colony-forming units, we queried whether administration of MAb to TNF- α on day 7 after infection would affect fungal burden. Recipients of MAb on day 7 after infection exhibited a significant elevation in levels of colony-forming units in the lungs and spleen (figure 1C and 1D), compared with levels in control mice ($P < .01$, ANOVA). We examined the survival of mice given MAb on the day of infection (day 0) or on day 3, 5, or 7 after infection. Mice administered MAb on day 0 or 3 manifested 100% mortality, and 60% of those given MAb on day 5 died. One hundred percent of infected control mice and recipients of MAb on day 7 survived (figure 1E).

In secondary histoplasmosis, administration of MAb to TNF- α was associated with a significant increase in the number of colony-forming units in the lungs and spleens, whether it was given on the day of infection or on day 3 or 5 ($P < .01$, ANOVA) (figure 2A and 2B). Separate groups of mice were monitored for survival, and only those that received MAb on day 0 or 3 manifested an altered survival profile. Infected control mice and those given MAb on day 5 all survived.

Phenotype of cells producing TNF- α . The finding that sustained production of TNF- α was required for optimal immunity led us to determine the cell population(s) producing this cytokine during the first week of infection (table 1). In both the lungs and spleens of mice with primary or secondary histoplasmosis, there was an increase in the number of TNF- α -positive cells from day 0 to day 7. The largest proportion of TNF- α -producing cells was T cells (table 1). From 40% to 70% of T cells of infected mice produced this cytokine.

TNF- α absence does not alter the number of IFN- γ -producing T cells. The finding that T cells were prominent generators of TNF- α , combined with the finding that sustained production of endogenous TNF- α was required for protective immunity, suggested that its absence might alter these cells' function. Mice were infected with *H. capsulatum* and given either rat IgG or MAb to TNF- α . One week after infection, the mice were killed, and the proportion of CD3⁺ IFN- γ -positive cells was determined. In both primary and secondary infection, the percentage and number of T cells from the lungs of TNF- α -neutralized mice expressing IFN- γ exceeded that of infected control mice (table 2). The number of non-CD3⁺ lung cells producing IFN- γ or TNF- α was similar between the 2 groups ($P > .05$, ANOVA) (data not shown).

Memory or activated T cell generation in TNF- α -neutralized mice. We explored the possibility that a deficiency in TNF- α would alter the number or proportion of CD3⁺ cells expressing a memory phenotype. Cells were labeled with CD44 and CD62L, and the percentage of cells was examined on day 7 after infection. The number of cells that bore a memory phenotype was significantly less in the lungs of TNF- α -neutralized mice with primary or secondary infection ($P < .05$ to $P < .01$, ANOVA) (figure 3). This finding was not true for spleens. In fact, in the spleens of mice with secondary histoplasmosis, the number of memory cells in the lungs of TNF- α -neutralized mice was greater than that in the lungs of control mice ($P < .01$, ANOVA).

We tested whether a deficiency of TNF- α altered activation of lung T cells in mice infected with *H. capsulatum*. Mice were infected with *H. capsulatum* intranasally and given either rat IgG or MAb to TNF- α . On day 7, lung leukocytes were examined for CD69, a T cell activation marker [28]. In primary infection, the mean $\pm$ SE number of lung CD3⁺CD69⁺ cells from mice given rat IgG ($1.2 \times 10^6 \pm 0.1 \times 10^6$; $n = 5$) did not differ from that from mice given MAb to TNF- α ($9.9 \times 10^5 \pm 1.0 \times 10^5$; $n = 5$) ($P > .05$, ANOVA). In secondary histoplasmosis, the number of CD3⁺CD69⁺ cells in the lungs of infected control mice ($2.8 \times 10^6 \pm 0.6 \times 10^6$; $n = 6$) did not differ from that of recipients of MAb to TNF- α ($2.6 \times 10^6 \pm 0.4 \times 10^6$; $n = 5$) ($P > .05$, ANOVA).

Bromodeoxyuridine staining of T cells from mice lacking TNF- α . To examine whether administration of MAb to TNF- α altered the proliferative capacity of T cells, infected mice were administered bromodeoxyuridine daily, and on day 7 the number of lung CD3⁺ T cells that incorporated it was assessed. In primary histoplasmosis, the number of bromodeoxyuridine-positive CD3⁺ cells from infected control mice ($1.0 \times 10^5 \pm 0.2 \times 10^5$; $n = 6$) did not differ from the number of positive cells from recipients of MAb to TNF- α ($2.0 \times 10^5 \pm 0.4 \times 10^5$; $n = 6$) ($P > .05$, ANOVA). In secondary infection, the number of CD3⁺ cells that incorporated bromodeoxyuridine ($1.6 \times 10^4 \pm 0.79 \times 10^4$; $n = 5$) was not significantly higher than that in mice that received MAb to TNF- α ($4.0 \times 10^3 \pm 0.4 \times 10^3$; $n = 5$) ($P > .05$, ANOVA).

Protection not conferred by T cells from mice given MAb to TNF- α . CD3⁺ T cells were isolated from the lungs of naive mice infected for 1 week and administered either rat IgG or MAb to TNF- α . Cells from each set were injected into separate groups of TCR $\alpha\beta^{-/-}$ mice; 2 days later, these mice were challenged with 2×10^6 yeasts intranasally. A control group received only diluent. Recipients of T cells from mice given MAb to TNF- α or diluent died of

infection by day 15, whereas a high proportion of recipients of cells from mice given rat IgG survived for 40 days ($P < .01$, log-rank test) (figure 4A). Transfer of T cells from the lungs of infected mice with secondary infection given rat IgG conferred a protective response ($P < .01$, log-rank test), whereas those from recipients of MAb to TNF- α did not (figure 4B).

An explanation for the failure of T cells from TNF- α -neutralized mice to confer protection was that fewer cells survived in organs of TCR $\alpha\beta^{-/-}$ mice. To address this issue, we transferred T cells from each group and infected TCR $\alpha\beta^{-/-}$ mice ($n = 3$) with 2×10^6 yeasts intranasally. On day 7 after infection, the mice were killed, and the number of T cells (detected using MAb to TCR $\alpha\beta$) was enumerated in the lungs and spleens. The number of transferred T cells obtained from infected control mice that were detected in the lungs (mean $\pm$ SE, $8.1 \times 10^4 \pm 2.2 \times 10^4$) and spleens (mean $\pm$ SE, $1.4 \times 10^6 \pm 0.6 \times 10^6$) was similar to the number in the lungs (mean $\pm$ SE, $8.5 \times 10^4 \pm 2.5 \times 10^4$) and spleens (mean $\pm$ SE, $2.4 \times 10^6 \pm 1.0 \times 10^6$) of recipients of T cells from TNF- α -neutralized mice ($P > .05$, ANOVA). Thus, the survival of T cells did not differ between the groups.

DISCUSSION

The finding that T cells constituted a high proportion of cells that expressed TNF- α and that sustained production of TNF- α was required for optimal expression of protective immunity in primary and secondary histoplasmosis led us to explore the importance of this cytokine in the generation of protective T cells. Our data demonstrate that the absence of this cytokine during the course of primary and secondary infection is associated with an irreversible defect in the protective capacity of T cells. The functional importance of this finding was firmly established by showing that T cells from mice given neutralizing MAb to this cytokine manifested an inability to confer protective immunity when transferred into TCR $\alpha\beta^{-/-}$ mice.

Protective immunity required the sustained presence of TNF- α in mice. Neutralization of the endogenous cytokine after infection was established led to an impaired protective immune response, as manifested by increased fungal burden and by the death of mice. These findings are somewhat surprising, given the fact that the production of TNF- α in the lungs after intrapulmonary challenge of mice results in a transient elevation (24 h) in cytokine release, followed by a sharp decline [13]. This pattern suggests that neutralization of TNF- α should affect protection only within the first 24 h of infection. On the contrary, the results indicate that even low levels of this cytokine are necessary for protection during acute infection. Alternatively, TNF- α that is membrane-bound could be active when soluble cytokine is low or absent.

Treatment with MAb to TNF- α on day 7 after infection did modify fungal burden but not survival. This finding contrasts with those for treatment with MAb on days 3 and 5, for which both fungal burden and mortality were affected. The most likely explanation for this result is that, by day 7, IFN- γ had achieved maximal levels, and this cytokine mediates protection in the absence of TNF- α [27].

CD3⁺ cells were a prominent source of TNF- α . Although TCR $\alpha\beta$ -positive cells constitute the highest proportion, natural killer T cells and TCR $\gamma\delta$ -positive cells also express CD3. We cannot exclude a role for these cells, but they have not been shown to modulate immunity to *H. capsulatum*. We were unable to determine if they constituted the largest number of cytokine-producing cells, because lung leukocytes were pooled to obtain sufficient numbers for phenotyping. The role played by T cells that produce TNF- α in murine histoplasmosis remains unclear. A recent study suggested that T cell-generated TNF- α was necessary for protection only against challenges with high inocula of *Listeria monocytogenes*, whereas phagocyte-derived cytokine was requisite for host defenses against any inoculum size [29]. In that model,

T cell-derived TNF- α appears to play a much more prominent role in the development of autoimmune conditions.

TNF- α expresses pleiotropic effects on immune and non-immune cells. This cytokine has been reported to be crucial for host resistance to a number of pathogens, including *M. tuberculosis*, *Leishmania major*, *Cryptococcus neoformans*, and *L. monocytogenes* [30-34]. The protective function of this cytokine in various infectious-disease models has been ascribed to enhancing nitric oxide synthesis, promoting granuloma formation, synergizing with IFN- α , and enhancing inflammation by regulation of chemokine production [32,35-37]. Less is known about the influence of this cytokine on T cell function.

We examined several properties of effector T cell function, including activation, memory acquisition, in vivo proliferation, and ability to transfer protection adoptively. In murine histoplasmosis, the production of IFN- γ and TNF- α appear to be independent [20]. Neutralization of endogenous TNF- α did not alter the number of IFN- γ -positive T cells in the lungs of mice. This result extends our previous finding that IFN- γ levels in homogenates of lungs from TNF- α -neutralized mice are elevated, compared with those in homogenates of lungs from infected control mice [20]. Thus, the absence of TNF- α does not diminish the production of another critically important cytokine in host resistance to this fungus. However, in primary infection but not secondary infection, TNF- α and IFN- γ must interact for protective immunity to be generated. In secondary infection, protection develops in the absence of IFN- γ but not TNF- α [16,20].

Activation and memory acquisition are vital properties of T cells, and TNF- α has been reported to influence these features [26,38-41]. Neutralization of this cytokine produced a decrement in the number of memory T cells but not activated (i.e., CD69⁺) cells. The decrease was not a complete elimination of these cells but rather a reduction of ~40%-50%. An explanation for the existence of memory cells is that there are TNF- α -independent mechanisms for acquisition. IL-15 and IL-7 are known to be central in generation of CD8⁺ memory T cells [40,41]. It might be expected that the number of memory cells would be increased in TNF- α -neutralized mice, because it is an extrinsic signal for apoptosis. Its absence may lead to a reduced number of apoptotic cells, including memory T cells [42-44]. By similar reasoning, a higher, rather than lower, number of activated cells might have been expected in TNF- α -depleted mice. The absence of TNF- α may impair the production of chemokines that are important in attracting memory cells [30,32].

TNF- α has been shown to enhance proliferative responses to stimuli that engage the TCR [25]. Therefore, we examined whether treatment with MAb to TNF- α altered proliferation in vivo. In the absence of this cytokine, there were no significant differences in the number of cells incorporating bromodeoxyuridine. Neutralization of TNF- α did not affect T cell division and, consequently, expansion.

Transfer of T cells from mice infected for 7 days into TCR $\alpha\beta^{-/-}$ mice mediated a protective immune response. This result strongly suggests that, in mice infected with *H. capsulatum*, protective T cell function is developed by the seventh day after infection and that these cells can function in a new host. Transfer of T cells from infected mice given MAb to TNF- α failed to induce a protective immune response in T cell-deficient mice. The failure of T cells from TNF- α -neutralized mice to recover function after explantation suggests that these cells acquire an irreversible defect in their protective capacity. Impaired correlates of T cell function also have been observed in mice infected with *C. neoformans* and administered MAb to TNF- α [30]. These mice do not control infection and lack a delayed-type hypersensitivity response to cryptococcal antigens. In that study, the precise function of T cells outside the host was not pursued.

In summary, we have demonstrated that T cells are a prominent source of TNF- α in mice infected with *H. capsulatum*. This cytokine must be present during the early period of infection for optimal expression of protective immunity. Fewer T cells from mice given MAb to TNF- α manifest a memory phenotype, and they appear to exhibit an irreversible defect in mediating protection. These results may have clinical import for patients receiving inhibitors of this cytokine for chronic inflammatory disease. Dysfunctional T cells may be one explanation for the emergence of histoplasmosis in these patients.

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Figure 1.

Host resistance to *Histoplasma capsulatum* requires sustained release of tumor necrosis factor (TNF)- α . Groups of mice ($n = 6$) were infected with 2×10^6 yeasts intranasally and given monoclonal antibody (MAb) to TNF- α on the day of infection (day 0) or on day 3 or 5 after infection. Colony-forming units in lungs (A) and spleens (B) were counted on day 7 after infection. A separate group of mice ($n = 6$) was treated beginning on day 7 after infection, and fungal burden in lungs (C) and spleens (D) was assessed on day 14 after infection. Data are means $\pm$ SEs. A survival curve is depicted in panel E. One of 2 experiments is shown. * $P < .05$; ** $P < .01$; # $P < .005$ (all by analysis of variance).

Figure 2.

Generation of tumor necrosis factor (TNF)- α and secondary histoplasmosis. Groups of mice ($n = 6$) were infected with 1×10^4 yeasts intranasally and rechallenged 8 weeks later with 2×10^6 yeasts intranasally. Separate groups received monoclonal antibody (MAb) to TNF- α on the day of infection (day 0) or on day 3 or 5 after infection. Colony-forming units in lungs (A) and spleens (B) were counted on day 7 after infection. Panel C depicts a survival curve. One of 2 experiments is shown. ** $P < .01$; # $P < .005$ (both by analysis of variance).

Figure 3.

No. of memory T cells in the lungs of mice given monoclonal antibody (MAb) to tumor necrosis factor (TNF)- α . Mice were infected with *Histoplasma capsulatum*, and lung CD4⁺ and CD8⁺ cells were analyzed for expression of CD62^{lo} and CD44^{hi}. Data are mean $\pm$ SE results for 6 mice. * $P < .05$; ** $P < .01$ (both by analysis of variance).

Figure 4.

T cells from tumor necrosis factor (TNF)- α -neutralized mice fail to transfer protection. Lung T cells were isolated from TNF- α -neutralized or infected control mice 1 week after infection. TCR $\alpha\beta^{-/-}$ mice ($n = 4-6/\text{group}$) were injected intraperitoneally with 1.25×10^6 cells/mouse, and survival was monitored. One of 3 experiments is shown. *A*, primary infection; *B*, secondary infection.

Table 1.

Phenotypes of tumor necrosis factor (TNF)- α -producing cells from lungs and spleens of mice infected with *Histoplasma capsulatum*.

Type of infection and source of cells, day of infection	Total no. (10^5) of TNF- α^+ cells, mean $\pm$ SE	F4/80	Gr-1	CD3 ϵ	CD11c
Primary lung					
0	ND	ND	ND	ND	ND
3	3.3 $\pm$ 0.3	16.6 $\pm$ 2.6	29.1 $\pm$ 12.9	43.7 $\pm$ 9.4	19.3 $\pm$ 5.1
7	6.4 $\pm$ 1.1	17.7 $\pm$ 2.0	8.6 $\pm$ 4.3	44.3 $\pm$ 1.6	23.0 $\pm$ 2.3
Primary spleen					
0	1.7 $\pm$ 0.3	7.8 $\pm$ 1.7	15.9 $\pm$ 1.4	62.7 $\pm$ 2.1	6.2 $\pm$ 1.5
3	5.8 $\pm$ 0.7	11.2 $\pm$ 1.3	16.0 $\pm$ 1.0	71.5 $\pm$ 2.6	11.5 $\pm$ 1.0
7	8.6 $\pm$ 2.1	6.7 $\pm$ 1.3	12.6 $\pm$ 1.6	72.7 $\pm$ 4.1	8.9 $\pm$ 1.8
Secondary lung					
0	1.3 $\pm$ 0.5	30.6 $\pm$ 1.6	26.6 $\pm$ 4.2	20.5 $\pm$ 3.2	26.8 $\pm$ 2.0
3	3.0 $\pm$ 0.3	21.0 $\pm$ 5.9	14.0 $\pm$ 3.5	40.7 $\pm$ 5.4	22.0 $\pm$ 6.0
7	3.1 $\pm$ 0.2	20.3 $\pm$ 3.7	13.3 $\pm$ 2.4	50.3 $\pm$ 1.2	19.7 $\pm$ 1.3
Secondary spleen					
0	2.2 $\pm$ 0.3	36.7 $\pm$ 4.4	21.8 $\pm$ 2.8	13.1 $\pm$ 1.7	27.5 $\pm$ 4.5
3	3.7 $\pm$ 0.3	17.7 $\pm$ 1.9	12.0 $\pm$ 1.3	61.1 $\pm$ 1.5	8.0 $\pm$ 1.3
7	4.2 $\pm$ 0.3	9.2 $\pm$ 1.3	14.3 $\pm$ 1.0	61.8 $\pm$ 1.5	11.2 $\pm$ 0.8

NOTE. Data are mean $\pm$ SE percentages of TNF- α -expressing cells, unless otherwise noted. Data for lungs are results from 5-6 pools of lungs, with 2-4 lungs for each data point. ND, none detected.

Table 2.

Absolute no. and percentage of CD3⁺ cells that synthesized interferon (IFN)- γ .

Infection, measure	Treatment		P
	Rat IgG	MAb to TNF- α	
Primary			
No.	1.23 $\pm$ 0.14	3.81 $\pm$ 0.26	.028
Percentage	13.90 $\pm$ 2.16	36.55 $\pm$ 8.12	.035
Secondary			
No.	43.56 $\pm$ 18.1	211.65 $\pm$ 2.94	<.01
Percentage	49.73 $\pm$ 6.06	51.22 $\pm$ 2.57	>.05

NOTE. Data are mean $\pm$ SE no. (10^5) or percentage of CD3⁺ IFN- γ -positive cells ($n = 5-6$ /group). MAb, monoclonal antibody; TNF- α , tumor necrosis factor- α .