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Regulation of Infection with *Histoplasma capsulatum* by TNFR1 and -2

Ruth Allendoerfer* and George S. Deepe, Jr. †

The concerted action of several cytokines is necessary for resolution of both primary and secondary infection with *Histoplasma capsulatum*. Among the soluble factors that contribute to tissue sterilization, TNF-α stands as a central mediator of protective immunity to this fungus. In this study, we explored the regulation of protective immunity by TNFR1 and -2. In primary pulmonary infection, both TNFR1^+/−^ and -2^−/−^ mice manifested a high mortality after infection with *H. capsulatum*, although TNFR1^+/−^ mice were more susceptible than TNFR2^−/−^ mice. Overwhelming infection in the former was associated with a pronounced decrement in the number of inflammatory cells in the lungs and elevated IFN-γ and TNF-α levels in the lungs. In contrast, IFN-γ levels were markedly decreased in TNFR2^−/−^ mice, and treatment with this cytokine restored protective immunity. Lung macrophages from both groups of knockout mice released substantial amounts of NO. Upon secondary infection, TNFR2^−/−^ mice survived rechallenge and cleared infection as efficiently as C57BL6/6 animals. In contrast, mice given mAb to TNFR1 succumbed to reexposure, and the high mortality was accompanied by a significant increase in fungal burden in the lungs. Both IL-4 and IL-10 were elevated in the lungs of these mice. The results demonstrate the pivotal influence of TNFR1 and -2 in controlling primary infection and highlight the differences between these receptors for regulation reexposure histoplasmosis. *The Journal of Immunology*, 2000, 165: 2657–2664.

Infection with the pathogenic fungus, *Histoplasma capsulatum* (Hc), is widely prevalent in the central and southeastern United States. The saprobic form of the organism is inhaled from the soil; upon entry into the lungs, the organism converts within hours to days into yeast cells that are the form responsible for the clinicopathological manifestations of histoplasmosis. Most acute infections are not clinically recognized, but the fungus does establish a dormant state in which it can be reactivated by insult to the integrity of the cell-mediated immune system (1).

Control of pulmonary infection with Hc is critically dependent on the release of cytokines from T cells and myeloid lineage cells. Among the endogenous cytokines that influence the outcome of both primary and secondary infection, TNF-α is a vital constituent of host defenses. Blockade of endogenous TNF-α is associated with overwhelming histoplasmosis both in a model using i.v. or intranasal (i.n.) inoculation of yeasts (2–5). The mechanisms underlying the inimical effects of mAb to TNF-α differ between primary and secondary infection. In the former, depletion of endogenous cytokine causes a depression in NO production. In contrast, administration of mAb to TNF-α induces an increase in IL-4 and IL-10 levels, thus causing a shift in the Th1/Th2 balance in secondary infection (4, 5).

The activities of TNF-α are dependent on the expression of two receptors, TNFR1 (p55) and TNFR2 (p75). TNF-α engagement of these two receptors either alone or in combination provokes a myriad of biological effects including apoptosis, cytotoxicity, T cell proliferation, shock, fever, and bone resorption (6–9). Because TNF-α is such a key component of host resistance to Hc, we examined the fate of mice that genetically lack either TNFR1 or TNFR2 following infection with this fungus. We demonstrated that the absence of expression of either TNFR1 or TNFR2 modulates protective immunity in primary infection. Although the outcome is similar, the cytokine profiles of the lungs of these mice differ. In secondary histoplasmosis, only TNFR1 is indispensable in host resistance.

**Materials and Methods**

**Mice**

TNFR1^+/−^, TNFR2^−/−^, and C57BL6/6 mice (5 wk of age) were purchased from The Jackson Laboratory (Bar Harbor, ME). All animal experiments were done in accordance with the Animal Welfare Act guidelines of the National Institutes of Health.

**Preparation of Hc and infection of mice**

Hc yeasts were prepared as described (10). To produce infection in naive mice, animals were infected i.n. with Hc yeasts in a 30-μl volume. For secondary histoplasmosis, mice were initially inoculated with 10^5^ yeasts i.n. Six to 8 wk later, previously exposed animals were rechallenged i.n. with 2.5 × 10^6^ yeasts.

**Organ culture for Hc**

Recovery of Hc was performed as described previously (10). The fungus burden was expressed as mean CFU per whole organ ± SEM. The limit of detection is 10^2^ CFU.

**Treatment with mAb or cytokine**

Endotoxin-free anti-TNFFR1 mAb (clone 55R-170, hamster IgG) was purchased from PharMingen (San Diego, CA). Mice were given 100 μg of mAb 24 h before infection and 3 and 7 days postinfection. Hamster IgG was purchased from Pierce (Rockford, IL). Recombinant murine IFN-γ (rmIFN-γ) was purchased from PeproTech (Rocky Hill, NJ). The cytokine was diluted in PBS to 5 μg/ml, and mice were injected with 1 μg i.p. daily.
for 2 wk. Subsequently, mice received 0.5 μg i.p. every other day until day 30.

Isolation of mononuclear cells from lungs

To isolate mononuclear cells from lungs, mice were sacrificed and lungs were flushed with 20 ml of HBSS by inserting a catheter into the right heart. The lungs were excised and teased apart with forceps and homogenized by sequential passage through 16-gauge, 18-gauge, and 20-gauge needles. Mononuclear cells were isolated by separation on a 40–70% Percoll (Pharmacia, Piscataway, NJ) gradient (10).

FACS analysis

Lung cells were adjusted to 5 × 10^7/200 μl in PBS containing 2% BSA and 0.02% sodium azide and stained with 0.5 μg of one of the following FITC- or PE-labeled mAbs (PharMingen): anti-CD4 (clone RM4-5), anti-CD8 (clone 53-6.7), anti-Ly-6G (Gr-1; clone RB6-8C5, which recognizes polymorphonuclear cells), or Mac-3 (clone M3/84, detects tissue macrophages (Mo)) or isotype-matched rat IgG mAb. The samples were washed and fixed in 2% paraformaldehyde until analyzed on a flow cytometer. Because cells other than blood lineage cells were present within the FACS analysis, the data were normalized to represent hematopoietic lineage cells within the gate by multiplying by the percentage of CD45^- cells. On average, the mean percentage of CD45^- cells exceeded 90% for all samples (10).

Reactive nitrogen intermediates (RNI) assay

Mononuclear cells from wild-type (wt) and TNFR1^−/− and -2^−/− mice (n = 6/group) were obtained on day 7 of primary infection and were seeded at 5 × 10^5 cells per well in 96-well plates in DMEM supplemented with 10% FBS. Nonadherent cells were removed after 2 h, and monolayers were stimulated with *Escherichia coli* O111:B4 LPS (Sigma, St. Louis, MO) and rmIFN-γ (1 μg and 100 ng/ml respectively). Supernatants were collected 48 h after seeding, filtered, and nitrite was measured by Griess reaction using Cayman’s nitrate/nitrite assay kit (Alexis, San Diego, CA).

Cytokine measurement

Lungs from infected mice (n = 5–6) were removed on either days 5 and 7 of primary infection or days 3, 5, and 7 of secondary infection. In primary infection, we chose to assay only on days 5 and 7 because our earlier work indicated that there is virtually no difference in any cytokine level between controls and experimental groups at day 3 (5). Tissue was homogenized in 10 ml of RPMI 1640, centrifuged at 1500 × g, filter sterilized, and stored at −70°C until assayed. Protein concentrations between groups did not vary for each day that was assayed (data not shown). The protein content of the homogenates ranged from 0.91 to 2.46 mg/ml among the various samples. Commercially available ELISA kits were used to measure IFN-γ, IL-4, GM-CSF, TNF-α (Endogen, Cambridge, MA), and IL-10 (R&D Systems, Minneapolis, MN). IL-12 was assayed by sandwich ELISA (PharMingen) specific for mouse IL-12 p70 protein. The sensitivity was >100 pg/ml.

Histology

Lungs were removed and tissues were fixed in 10% formalin and embedded in paraffin blocks. Sections (5 μm) were stained with hematoxylin and eosin. Analysis of the sections was performed in a “blinded” fashion.

Statistics

Student’s t test was used to compare groups if the data achieved normality, otherwise the Wilcoxon rank sum test was used. Survival data was analyzed using the log rank test.

Results

Fate of TNFR1^−/− and -2^−/− mice infected with Hc

TNFR1^−/− and -2^−/− mice and wt controls were exposed i.n. to 2.5 × 10^6 yeasts, which is a sublethal inoculum in wt (10, 11). All TNFR1^−/− and -2^−/− mice succumbed to Hc infection within a nearly identical time frame, whereas 100% the infected controls survived for 45 days (p < 0.001). In companion experiments, we determined if survival could be correlated with inoculum size. Groups of TNFR1^−/− and -2^−/− mice and wt controls were challenged i.n. with 10^4 or 10^5 Hc yeasts and followed for up to 45 days. All TNFR2^−/− and wt mice survived for 45 days (p > 0.05). At this time, mice were sacrificed and assessed for fungal burden. Lungs and spleens from both groups contained <200 CFU, the limit of detection. In contrast, all TNFR1^−/− mice (n = 8/group) expired following exposure to either inoculum. The mean survival time was 25 ± 4 days (p < 0.001) and 20 ± 5 days (p < 0.001) for the 10^4 and 10^5 inocula, respectively.

To ensure that the knockout mice were not becoming ill merely from exposure to the environment, groups (n = 5) of uninfected TNFR1^−/− and -2^−/− mice were observed for 30 days. None of the mice demonstrated signs of illness such as weight loss, huddling, or ruffled fur.

We determined if the absence of TNFR1 or -2 influenced the course of secondary infection. TNFR2^−/− mice and wt controls were exposed to 10^5 yeasts i.n. and 8 wk later challenged with 2.5 × 10^6 yeasts. TNFR1^−/− mice were extremely susceptible to Hc; even treatment with amphotericin B, 5 mg/kg, three times per week failed to sterilize tissues, and mice succumbed to overwhelming histoplasmosis. Accordingly, C57BL/6 mice were immunized with 10^4 yeasts, and they were given 100 μg mAb to TNFR1 i.p. 24 h before infection and 3 and 7 days postinfection. This dosage of mAb to TNFR1 was sufficient to block the effects of LPS-mediated death in mice (Ref. 12 and data not shown). Upon reexposure to Hc, all mice administered mAb to TNFR1 expired, whereas infected controls and TNFR2^−/− mice survived for 45 days (Fig. 1B).

Fungal recovery in TNFR1^−/− and -2^−/− mice

To assess if mortality of knockout mice or mAb to TNFR1 recipients was associated with a higher fungal burden, CFU were determined in lungs and spleens of wt, TNFR1^−/−, and -2^−/− mice.
exposed to $2.5 \times 10^6$ yeasts. At day 7 of primary infection, the number of CFU in the lungs of either knockout strain was dramatically higher ($p < 0.001$) than in lungs of wt controls (Fig. 2A). The burden in lungs of TNFR1$^{-/-}$ mice exceeded ($p < 0.001$) that of TNFR2$^{-/-}$ mice. In spleens, fungal recovery differed ($p < 0.01$) between wt and TNFR1$^{-/-}$ and between TNFR2$^{-/-}$ and wt mice ($p < 0.001$) (Fig. 2B).

In secondary infection, Hc recovery was similar in TNFR2$^{-/-}$ mice and infected controls at days 7 and 14 (Fig. 3, A and B). On day 45, all survivors were sacrificed, and lungs and spleens were assessed for fungal burden. No CFU (<200) were detected from either TNFR2$^{-/-}$ or wt animals. The burden of Hc in lungs and spleens of mice administered mAb to TNFR1 at day 7 of infection was markedly elevated ($p < 0.01$) when compared with infected controls (Fig. 3, C and D).

Phenotypic analysis of lung mononuclear cells

Lung cells from wt, TNFR1$^{-/-}$, and TNFR2$^{-/-}$ mice were analyzed 7 days postinfection to determine whether there was perturbation in the infiltration of cell populations into mice. There was a striking decrement ($p < 0.01$) in the total number of lung cells from TNFR1$^{-/-}$ mice as compared with wt or TNFR2$^{-/-}$ animals (Table I). Nevertheless, the proportion of cells expressing CD4, CD8, Gr-1, or Mac-3 did not differ among the groups.

The number of inflammatory cells recovered from rechallenged mice given mAb to TNFR1 was significantly less ($p < 0.01$) than infected controls. Similar to primary infection, the proportion of cell subpopulations was similar between the two groups (Table I).

Histopathology of lungs of mice infected with Hc

Lung tissue of infected wt, TNFR1$^{-/-}$, and -2$^{-/-}$ mice was examined at week 1 of primary infection to determine whether the inflammatory response to Hc differed among these three groups. Mild to moderate perivascular lymphoid cuffing was observed in lung parenchyma of infected controls. An admixture of neutrophils and granulomatous inflammation was present involving between 30% and 60% of the lung tissue.

In lungs of TNFR1$^{-/-}$ mice, there was a severe pneumonitis with destruction of nearly all the pulmonary architecture. Cellular infiltration, which was less than in controls, consisted predominantly of neutrophils and mononuclear phagocytes. Lymphocyte cuffing was limited to the perivascular and peribronchiolar regions. Analysis of lungs from TNFR2$^{-/-}$ animals demonstrated a severe pneumonitis with 50–75% destruction of the normal architecture.

![FIGURE 3. Fungal recovery from lungs (A) and spleens (B) of Hc-immune wt and TNFR2$^{-/-}$ mice infected for 7 and 14 days after i.n. inoculation with $2.5 \times 10^6$ yeasts. Fungal burden in lungs (C) and spleens (D) of Hc-immune mice administered mAb to TNFR1. Results are shown for day 7 of infection. **, $p < 0.01$. Data are expressed as mean $\pm$ SEM of six mice per group. One of two experiments is illustrated.](http://www.jimmunol.org/)
Table 1. Cell populations in lungs of Hc-infected wt, TNFR1−/−, TNFR2−/−, and mAb to TNFR1-treated mice*  

<table>
<thead>
<tr>
<th>Challenge</th>
<th>Cell Population</th>
<th>Mean Cell No. (± SEM) × 10^6</th>
<th>wt</th>
<th>TNFR1−/−</th>
<th>TNFR2−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total cells</td>
<td>3.24 ± 1.9</td>
<td>0.68 ± 0.32</td>
<td>3.36 ± 0.52</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CD4+</td>
<td>0.26 ± 0.15 (7.2)*</td>
<td>0.04 ± 0.03 (6.8)</td>
<td>0.28 ± 0.19 (8.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CD8+</td>
<td>0.18 ± 0.08 (5.6)</td>
<td>0.04 ± 0.02 (6.1)</td>
<td>0.22 ± 0.12 (6.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gr-1+</td>
<td>1.64 ± 0.48 (47.5)</td>
<td>0.33 ± 0.12 (52.4)</td>
<td>1.72 ± 0.65 (40.2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mac-3+</td>
<td>1.24 ± 0.41 (50.6)</td>
<td>0.27 ± 0.15 (35.9)</td>
<td>1.34 ± 0.33 (32.3)</td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>Total cells</td>
<td>7.32 ± 1.34</td>
<td>2.90 ± 0.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CD4+</td>
<td>3.45 ± 1.06 (50.7)</td>
<td>1.41 ± 0.67 (52.3)</td>
<td></td>
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<tr>
<td></td>
<td>CD8+</td>
<td>1.78 ± 0.68 (24.1)</td>
<td>0.73 ± 0.18 (22.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gr-1+</td>
<td>0.32 ± 0.07 (4.6)</td>
<td>0.16 ± 0.02 (6.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mac-3+</td>
<td>0.55 ± 0.11 (8.1)</td>
<td>0.29 ± 0.16 (7.6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Lungs of mice (n = 6–8) were prepared on day 7 of infection as described in Materials and Methods. The percent of cells expressing a surface phenotype was determined by FACS. Analysis was normalized to the CD45+ population. The number of cells was calculated by multiplying the total number of cells by percent of cells expressing a phenotype.

In primary infection, the number of total cells and each cell subset isolated from TNFR1−/− mice was significantly less (p < 0.01) than cells from controls or TNFR2−/− mice. There was no statistical difference (p > 0.05) between controls and TNFR2−/− mice. In secondary infection, the number of total cells and each cell subset isolated from mice given mAb to TNFR1 was less (p < 0.01) than controls.

* Numbers in parentheses indicate mean percentage of cells expressing a phenotype.

The infiltrate consisted primarily of neutrophils and mononuclear phagocytes, but lymphocyte cuffing of perivascular and peribronchial regions was less prominent than in TNFR1−/− mice. Granulomatous inflammation was present in both groups of knockout mice.

Lung tissue from rechallenged mice was examined on day 7 of infection. In mice given hamster IgG, there was moderate to severe peribronchial infiltration of a similar number of lymphocytes and macrophages. Granulomatous inflammation involved 20–30% of lung tissue. In mice given mAb to TNFR1, fewer cells were present in lungs, but there was an equal admixture of lymphocytes and mononuclear phagocytes. Neutrophils were sparse. The extent of granulomatous inflammation ranged from 30 to 50% of the pulmonary parenchyma.

Cytokine analysis of lungs from Hc-infected animals

Wt, TNFR1−/−, and TNFR2−/− mice were infected with Hc, and lungs were assayed for the presence of IFN-γ, TNF-α, GM-CSF, and IL-4, -10, and -12 on days 5 and 7 of infection. These cytokines were selected because of their known immunomodulatory effects on the course of Hc infection (4, 5, 11, 13, 14). Lungs of TNFR1−/− mice contained exceedingly higher levels of IFN-γ than those of infected controls or of TNFR2−/− mice (p < 0.005) on days 5 and 7. IFN-γ levels in lungs of TNFR2−/− mice were substantially lower than that found in infected controls (p < 0.01) on both time points (Fig. 4). TNF-α levels were higher in TNFR1−/− mice than in infected controls or TNFR2−/− mice only on day 5 (p < 0.01). GM-CSF, IL-12, IL-4, and IL-10 levels did not vary significantly among the groups (p > 0.05).

In secondary infection, cytokine analysis was performed in mice given mAb to TNFR1 because they succumbed to rechallenge with Hc. Levels of TNF-α and GM-CSF did not differ (p > 0.05) from that of infected controls. IFN-γ levels in lungs of mice given mAb to TNFR1 were significantly greater (p < 0.05) than infected controls on days 5 and 7. IL-4 and IL-10 levels also were increased (p < 0.01) on days 5 and 7 postinfection (Fig. 5).

RNI production by lung MΦ from TNFR1−/− and -2−/− mice

Generation of NO is crucial for the development of protective immunity in primary infection with Hc (5, 13). To determine whether mortality in TNFR1−/− and -2−/− mice was accompanied by a decrement in release of RNI, lung MΦ from mice infected for 7 days were incubated with or without LPS and IFN-γ, and NO was measured. Cells from each group of mice released NO above that found in unstimulated cells. The responses by TNFR2−/− lung MΦ stimulated with LPS plus IFN-γ exceeded (p < 0.01) those of cells from wt or TNFR1−/− mice (Table II). A comparison of NO release by TNFR1−/− and wt MΦ revealed no differences (p > 0.05).

Can exogenous IFN-γ alter the course of histoplasmosis in TNFR2−/− mice?

Endogenous IFN-γ is requisite for control of primary infection with Hc in mice (11, 13). The depressed levels of IFN-γ in TNFR2−/− mice raised the possibility that this deficiency contributed to the aggressive course of infection. Therefore, we sought to determine whether exogenous administration of this cytokine might restore the protective immune response. TNFR2−/− mice were administered 1 μg of rmIFN-γ i.p. or an equal volume of PBS daily. At day 7 of infection, mice were sacrificed and CFU were determined. There were no differences (p > 0.05) in CFU in lungs or spleens between the two groups (Fig. 6). Separate groups of mice treated with IFN-γ or diluent were followed for survival. All mice receiving diluent died by day 14. Administration of rmIFN-γ prolonged survival over a 30-day observation period; 67% of them survived (p < 0.01). At day 30, the survivors were sacrificed, and lungs and spleens were assessed for Hc CFU. The number of CFU in lungs ranged from 3 × 10^2 to 2 × 10^3 CFU and in spleens from 10^2 to 10^3 CFU.

Discussion

Several reports have established a central role for endogenous TNF-α in the generation of a protective immune response in mice exposed to Hc either i.v. or i.n. (2–5, 15). In this study, we have examined the influence of TNFR in regulating the generation of a protective immune response in primary and secondary pulmonary infection. The absence of either TNFR1 or -2 abrogated effective protective immune response in primary and secondary pulmonary infection. The relative significance of the two
receptors could only be discriminated when we challenged mice with very low inocula of this fungus. Unlike the findings in other experimental models, the data indicate that signaling through TNFR2 is critically important for limiting Hc.

Resistance to primary Hc infection requires the development of a Th1-type response that includes production of IL-12 and IFN-γ (10, 11, 13, 15). We have demonstrated that neutralization of TNF-α did not diminish generation of either of these cytokines. In fact, IFN-γ levels in the lungs of mice given mAb to TNF-α were elevated compared with controls (5). Unexpectedly, the absence of TNFR1 or TNFR2 resulted in discordant generation of IFN-γ levels in the lungs. TNFR1−/− infected with Hc produced markedly elevated levels of this cytokine as compared with controls. Likewise, TNFR2−/− mice infected with L. major develop a Th1 response (18). In contrast, TNFR2−/− mice failed to make an appropriate Th1 response; IFN-γ levels were markedly diminished compared with controls and to TNFR1−/− animals, but the blunted levels of IFN-γ were not caused by poor production of IL-12. The biological significance of impaired IFN-γ levels was attested to by demonstrating that rmIFN-γ treatment of TNFR2−/− mice restored protective immunity to 67% of animals. The salutary effects of this cytokine required prolonged treatment because no differences in CFU were apparent between the two groups at day 7 of infection.

Recent evidence suggests that TNF-α can promote activation of the IFN-γ signaling complex. Elaboration of IFN-γ is dependent on activation of Jak-1 and -2 and phosphorylation of STAT1 (23, 24). TNF-α treatment increases Jak-2 kinase activity and STAT1 phosphorylation (25). Murine TNF-α induces Jak-1 and -2 activity and phosphorylation of STAT1, -3, -5, and -6, and the bioactivity of TNF-α is mediated by TNFR1 (26). In Hc infection, TNFR2 seems to be key in the generation of the appropriate signals to synthesize IFN-γ. Although the nature of this interaction remains to be elucidated, the current findings support the contention that there is a link between TNFR and IFN-γ production.

Hc-immune mice that were treated with mAb to TNF-α manifested increased susceptibility to infection in conjunction with elevated levels of IL-4 and IL-10 in their lungs (5). The inimical consequences of these increases was mitigated by treating TNF-α-neutralized mice with mAb to IL-4 and IL-10 (5). The present findings define the influence of TNFR1 in both the regulation of protective immunity and the generation of elevated levels of IL-4 and IL-10 in secondary infection. Blockade of TNFR1 by mAb led to excess mortality and increased levels of IL-4 and -10 in the lungs of
rechallenged mice. One potential explanation for the dichotomous influences of TNF-α and IL-4 and -10 in Hc infection is modulation of apoptosis. In certain models of mycobacterial infection, apoptosis promotes host resistance (27–29). A postulated mechanism is that uptake of apoptotic bodies containing viable organisms by uninfected phagocytes triggers a signal cascade that promotes growth inhibition. IL-10 and IL-4 oppose the apoptosis-inducing effect of TNF-α (28, 29). Conceivably, the lack of TNF-α or an inability to signal through TNFR1 combined with increases in IL-4 and IL-10 reduce apoptosis in Hc infection, thus exacerbating infection.

TNF-α exerts proinflammatory activity either directly or through regulation of chemokine synthesis (30, 31), and expression of TNFR may influence the character of the inflammatory response. TNFR1<sup>−/−</sup>/2<sup>−/−</sup> mice exhibit reduced inflammation in immune complex uveitis (32). Conversely, TNFR1<sup>−/−</sup> mice mount a vigorous inflammatory response in Escherichia coli pneumonia (33). Analysis of inflammatory cell constituents recovered from lungs of TNFR1<sup>−/−</sup> mice or those given mAb to TNFR1 revealed a marked depression in the absolute number of cells as compared with controls or TNFR2<sup>−/−</sup> mice. However, the proportion of cell subpopulations was similar between controls and TNFR1<sup>−/−</sup> mice or those given mAb to TNFR1. These results were surprising in light of our previous findings in which mice treated with mAb to TNF-α did not manifest any pronounced differences in either the numbers or proportions of cells expressing CD4<sup>+</sup>, CD8<sup>+</sup>, Gr-1<sup>+</sup>, or in the number of Mφ (5). These data clearly expose an apparent dichotomy between the ability of TNFR1 and TNFR2 to evoke inflammation.

### Table II. NO production by lung Mφ from wt, TNFR1<sup>−/−</sup>, and TNFR2<sup>−/−</sup> mice<sup>a</sup>

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Stimulus&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Mean μM NO&lt;sub&gt;2&lt;/sub&gt; (±SEM)&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt</td>
<td>–</td>
<td>40.5 ± 4.9</td>
</tr>
<tr>
<td>TNFR1&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>–</td>
<td>25.9 ± 4.7</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>72.3 ± 5.0</td>
</tr>
<tr>
<td>TNFR2&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>–</td>
<td>34.9 ± 5.7</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>86.9 ± 4.1</td>
</tr>
</tbody>
</table>

<sup>a</sup> NO was measured as described in Materials and Methods. The data are expressed as mean μM NO<sub>2</sub> ± SEM. Results are from eight mice per group.

<sup>b</sup> The stimulus was E. coli O111:B4 LPS (1 μg) plus IFN-γ (100 ng/ml).

<sup>c</sup> The mean value for TNFR2<sup>−/−</sup> mice exceeded (<i>p</i> < 0.01) that found for controls and TNFR1<sup>−/−</sup> mice. There was no statistical difference (<i>p</i> > 0.05) between controls and TNFR1<sup>−/−</sup> mice.
TNF-α is reported to be necessary in some models for generation of granulomatous inflammation (34, 35). We have demonstrated that in TNF-α-neutralized mice, the character of the inflammatory response to Hc was similar to that of infected controls as measured histopathologically (5). In primary infection, analysis of the lung pathology in TNFR1−/− and 2−/− mice revealed the presence of granulomatous inflammation, although the extent of the inflammation was more pronounced than in infected controls. Similarly, the inflammatory response in Hc-immune mice given mAb to TNFR1 was similar to that of controls. Thus, the absence of a functional TNFR did not perturb the development of granulomatous inflammation in response to this fungal pathogen.

There exists a paradox between the severity of the lung pathology in TNFR1−/− mice or those given mAb to TNFR1 and the reduced number of inflammatory cells. The quantity of obliterated lung tissue was not commensurate with the number of cells recovered from lungs. The altered architecture may have been a consequence of cells that express a heightened activation state in an attempt to control the increased burden of Hc. The toxic products of neutrophils and mononuclear phagocytes (e.g., oxygen radicals and/or nitrogen intermediates) may harm the host more than Hc. This contention is supported by the finding that Hc survives the respiratory burst (36). An additional possibility is that the increased number of Hc in the TNFR1−/− mice or those given mAb to TNFR1 contributed to the disrupted lung architecture.

Generation of NO is a major host resistance mechanism for primary but not secondary infection with Hc and TNF-α is requisite for synthesis of optimal amounts (4, 5). Hence, we determined if production of NO was modulated in TNFR1−/− or 2−/− mice. Lung Mϕ from either knockout strain released amounts of NO comparable to or exceeding those of infected controls. Others have found that NO production is not impaired in TNFR1−/− or TNFR1/2−/− mice who are chronically infected with T. gondii or who are exposed to L. major (18, 19). Conversely, in acute toxoplasmosis, NO release is diminished in TNFR1−/− mice (20). The findings herein were unexpected because blockade of TNF-α was accompanied by diminished NO generation by alveolar Mϕ in response to LPS plus IFN-γ (5). Moreover, TNFR2−/− mice release NO in the absence of a vigorous IFN-γ response. Collectively, the results suggest that impaired NO production requires either the absence of both TNFR or a lack of appropriate signals for these receptors or both.

One explanation for the differences in NO production between mice treated with mAb to TNF-α and TNFR1−/− or TNFR2−/− mice is that alveolar Mϕ were assayed in the former and parenchymal Mϕ were analyzed in the knockout mice (5). This consideration is unlikely because parenchymal Mϕ from Hc-infected mice given mAb to TNF-α also manifest depressed NO release. For example, the mean µM of NO2 from lung leukocytes of 1-wk-infected mice (n = 6) given rat IgG stimulated with LPS plus IFN-γ (48.3 ± 6.2) was more (p < 0.01) than that from cells of infected mice (n = 6) treated with mAb to TNF-α (8.3 ± 1.7) (R. Allendoerfer and G. Deepe, unpublished observations).

In summary, we have delineated the relative importance of TNFR1 and -2 on control of pulmonary histoplasmosis. Our data demonstrate that the absence of either receptor renders mice susceptible to primary infection. Differences in the mechanisms of susceptibility to primary infection were identified between TNFR1−/− and TNFR2−/− mice. The former manifested a marked impairment in the quantity of inflammatory cells within the lungs.
Overwhelming Hc infection in naive TNFR2−/− was associated a reduced generation of IFN-γ. Only TNFR1 is required for maintenance of secondary immunity. Blockade of this receptor results in disease exacerbation and increases in IL-4 and -10. These findings provide a greater understanding of the influence of TNF-α on host resistance to Hc.

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References