The Role of Recombinant Murine IL-12 and IFN-γ in the Pathogenesis of a Murine Systemic Candida albicans Infection


http://www.jimmunol.org/content/160/1/284

References
This article cites 46 articles, 22 of which you can access for free at:
http://www.jimmunol.org/content/160/1/284.full#ref-list-1

Subscription
Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
The Role of Recombinant Murine IL-12 and IFN-γ in the Pathogenesis of a Murine Systemic Candida albicans Infection

Liz M. Lavigne, Lisa R. Schopf, Charles L. Chung, Rich Maylor, and Joseph P. Sypek

Studies on murine candidiasis suggest that resistance to disease is linked to a Th1 response and production of IFN-γ, while failure to elicit protection is associated with a Th2 response and production of IL-4 and IL-10. Experimental infection of C57BL/6 mice, IL-12 treatment of these mice, or both infection and IL-12 treatment resulted in a characteristic Th1 cytokine mRNA profile as measured by quantitative competitive PCR. Specifically, little or no IL-4 transcripts were detected, while IFN-γ message was elevated, particularly with IL-12 treatment. Despite its role in driving increased IFN-γ expression and production, IL-12 treatment, paradoxically, promoted disease progression in our model. Therefore, we examined the effect of IFN-γ neutralization on IL-12-induced susceptibility to infection. None of the systemically infected mice receiving IL-12 alone survived, while IL-12- and anti-IFN-γ-treated mice had a 70% survival rate, similar to that after infection alone. These results suggested that IFN-γ induced by IL-12 treatment contributed to lethality. However, in separate studies, IFN-γ knockout mice were more susceptible to infection than their wild-type counterparts, suggesting that IFN-γ is required for resistance. Nonetheless, infected IFN-γ knockout mice treated with recombinant murine IL-12 exhibited enhanced resistance, suggesting that the toxicities observed with IL-12 are directly attributable to IFN-γ and that an optimal immune response to Candida infections necessitates a finely tuned balance of IFN-γ production. Thus, we propose that although IFN-γ can drive resistance, the overproduction of IFN-γ during candidiasis, mediated by IL-12 administration, leads to enhanced susceptibility. The Journal of Immunology, 1998, 160: 284–292.

Among the yeast that constitute the normal microbiota of the skin, mucous membranes, and gastrointestinal tract, the species Candida albicans is most frequently associated with opportunistic infections in man. As a disease entity, candidiasis is prevalent in individuals receiving corticosteroids, cytotoxic or immunosuppressive drugs, or radiation therapy and in those with hematologic malignancies or AIDS (1–3). Such observations indicate that intact cell-mediated immunity is essential for resistance to this fungal infection.

The development of distinct Th responses and cytokine profiles in mice has been shown to critically influence the successful outcome of host defense against microbial and parasitic pathogens (4, 5). While innate defense mechanisms (primarily granulocyte and macrophage mediated) appear to be the initial means by which infections caused by Candida albicans are contained, clearance of infection and long term immunity appear to be determined by the emergence of T cell-mediated immune responses (6). Studies using a systemic model of infection suggest that protection is associated with the expression of a Th1 response, while nonprotective immunity is correlated with a Th2 response (7–9). In particular, administration of anti-IL-4 Ab or an IL-4-soluble receptor antagonist at the time of infection results in a protective Th1 immune response (10, 11), while, conversely, anti-IFN-γ administration leads to a nonprotective Th2 response (7, 12).

Additionally, studies suggest that development of this protective immunity against C. albicans is linked to the production of endogenous IL-12 during infection (13, 14). IL-12 is a heterodimeric cytokine that exhibits a number of bioactivities that may modulate infectious disease progression, including enhancing NK and T cell cytotoxicity, modulating T cell proliferation, and potentiating Th1 differentiation (15, 16). Moreover, IL-12 influences the production of other immunoregulatory cytokines, particularly IFN-γ, that are thought to help generate protective immunity and to be necessary for microbial killing by macrophages and neutrophils (17, 18).

To gain insight into the functional role of IL-12 in the in vivo development of the cellular immune response, in the present study we examined the effect of exogenous IL-12 administration on the course and outcome of systemic C. albicans infection in mice. We observed that exogenous IL-12, rather than exerting beneficial activity against infection, paradoxically promoted disease progression. We provide evidence that this effect was mediated through the ability of IL-12 to enhance the expression and the production of IFN-γ. We suggest that IFN-γ plays a critical role in pathogenesis despite the evidence that Th1 responses are thought to contribute to protective immunity in this model.

Materials and Methods

Animals, yeast, and infections

Female C57BL/6 wild-type and IFN-γ knockout mice (The Jackson Laboratory, Bar Harbor, ME), ranging in age from 6 to 8 wk and in weight from 18 to 20 g, were purchased and maintained under American Association for the Accreditation of Laboratory Animal Care-approved conditions. C. albicans (strain 30682, American Type Tissue Collection, Rockville, MD) was obtained as a freeze-dried stock. The culture was rehydrated and grown in a bactopeptone broth (Difco, Detroit, MI) for 1 day at room temperature, then streaked onto a bactopeptone agar plate and grown for 4 or 5 days at ambient temperature. Plates were stored at 4°C. Before infection, a colony from a selected plate was grown in bactopeptone broth overnight, washed three times in physiologic saline (Abbott Laboratories, North Chicago, IL), and measured by spectrophotometry at 540 nm to determine the yeast concentration. For sublethal and lethal challenge...
lethal challenge and again the following day. For neutralization studies, on day 0 and administered IL-12 at a dose range of 0.001 to 0.11 given i.p. in a 0.2-ml volume. In dose-response studies, mice were infected MRB7292 and 021693–1.1) was diluted in sterile physiologic saline and or saline i.p. on days 0 through 4 via a single bolus dose. In all other studies, mice were infected via bolus tail vein injection of 0.1 ml with 5 x 10^6 CFU/mouse of G. albicans, respectively. Survival was monitored for 15 to 30 days.

**Test and control articles**

Recombinant murine IL-12 (rmIL-12), produced at Genetics Institute (lots MR7292 and 021693–1.1) was diluted in sterile physiologic saline and or saline i.p. on days 0 through 4 via a single bolus dose. In all other experiments, mice were chronically infected, then injected with physiologic saline or with 0.1 μg/mouse rmIL-12 i.p. immediately after this sublethal challenge and again the following day. For neutralization studies, endotoxin-free rat IgG1 mAb to IFN-γ, clone XMG1.2 (American Type Tissue Collection, catalogue no. HB10648) was administered via i.p. bolus injection at 0.5 mg/mouse in a volume of 0.2 ml for 2 days. Rat IgG1 (Sigma Chemical Co., St. Louis, MO; catalogue no. I-4131) was rendered endotoxin free by Triton X-114 phase separation (19) and used as a control article for anti-IFN-γ studies. All articles were diluted to appropriate concentrations using sterile physiologic saline.

**Histology methods**

Mice were euthanized on day 3 of chronic infection. Kidneys were collected, cut cross-sectionally, and fixed in 10% neutral buffered formalin. Tissue samples were embedded in paraffin, then cut into 5-μm sections and floated onto glass slides. Sections were stained with hematoxylin and eosin (H&E) for morphologic examination or with a periodic acid solution for morphologic examination or with a periodic acid solution for morphologic examination. Peritoneal exudate cells were obtained by lavage with 5 ml of FBS-free culture medium, then plated in 96- and 24-well plates (Costar, Cambridge, MA) in the presence of medium alone, supplemented with nitric oxide synthase.

<table>
<thead>
<tr>
<th>Table 1. Reaction conditions for quantitative, competitive reverse-transcription PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene</td>
</tr>
<tr>
<td>G3PDH Upper</td>
</tr>
<tr>
<td>Lower</td>
</tr>
</tbody>
</table>

a Cutoff values shown are less than or equal to the copy number of the respective gene per 1000 copies of G3PDH (spleen only). NA, not applicable.

Con A (Sigma) at a final concentration of 2.5 μg/ml, or heat-killed Candida at 5 x 10^6 CFU/ml. Splenocytes and lymph node cells were plated at 4 x 10^5 cells/well in 96-well plates and at 2.2 x 10^5 cells/well in 24-well plates. Peritoneal cells were plated at 10^7 cells/well in 96-well plates or at 5.5 x 10^5 cells/well in 24-well plates. All cell cultures were incubated at 37°C in an atmosphere of 5% CO2. Supernatant fluids were harvested at 24 and 48 h and stored at −80°C.

**Cytokine assays**

Cytokine levels in sera or culture supernatant fluids were assessed by ELISAs. Commercially available kits were used to assay for IFN-γ and IL-10 (Endogen, Cambridge, MA). Reagents for IL-4 assays were obtained from PharMingen (San Diego, CA) and were used as follows. Dynatech Immulon-4 ELISA plates (Fisher Scientific, Fairlawn, NJ) were coated with 0.5 μg/ml cytokine-specific capture Ab overnight at 4°C, washed four times with PBS and 0.05% Tween-20, and blocked for 30 min with PBS and 2% BSA at 37°C. After a single washing step, the standards and supernatant fluids were added to the wells and incubated for 2 h at 37°C or overnight at 4°C. Wells were then washed four times and incubated with detector Ab at a concentration of 0.5 μg/ml. The ELISA plates were amplified and developed, after a final wash, using the Vectastain ABS kit (Vector Laboratories, Burlingame, CA) according to the manufacturer’s instructions.

**Nitrite/nitrate determinations**

Nitrate and nitrite concentrations were used as a relative measure of nitric oxide (NO) synthesis (22) and were measured in the sera using a NO analyzer (Sievers Instruments, Inc., Boulder, CO) according to the manufacturer’s instructions.

**Quantitative competitive PCR (QC-PCR)**

Total RNA was purified from spleens previously snap-frozen in liquid nitrogen. The tissue was thawed in 5.7 M guanidine isothiocyanate (Life Technologies, Gaithersburg, MD), then homogenized with a Tissuemizer electric tissue homogenizer (Tekmar Co., Cincinnati, OH). RNA was isolated from the homogenate by cesium chloride centrifugation followed by phenol chloroform extraction and ethanol precipitation (23) and were stored at −80°C. Single-stranded cDNA was generated from the RNA with oligo(dT) priming and avian myeloblastosis virus reverse transcriptase (Promega Corp. Madison, WI) according to the manufacturer’s protocol. The resulting cDNA was diluted 10-fold for a working solution and used as a template for gene-specific QC-PCR against nonhomologous MIMIC fragments (Clontech Laboratories, Palo Alto, CA). Amplification reactions contained cytokine-specific 5′ and 3′ oligonucleotide primers obtained from Clontech or synthesized at Genetics Institute and are shown in Table I. To control for experimental artifacts, each calculation was normalized to mRNA levels of a housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (G3PDH). Briefly, cDNA samples were added to a 4-fold dilution series of MIMICS with reaction buffer and PCR primers, then layered with mineral oil. Reactions were mixed and heated at 94°C for 3 min, then
cooled to 40°C. AmpliTaq DNA polymerase (Perkin-Elmer, Branchburg, NJ) was quickly added, and the products were amplified under the following conditions: initial denaturation at 94°C for 1 min, followed by 25 to 35 cycles of denaturation (94°C for 1 min; annealing temperatures are listed in Table I), and extension at 72°C for 7 min. Amplifications were performed in an Omnigene thermal cycler (Hybaid, Middlesex, U.K.). Resulting amplified products were analyzed by 1.2% agarose gel (FMC, Rockland, ME) electrophoresis followed by ethidium bromide staining.

Statistical analysis

Survival data were tabulated and analyzed using the Fisher’s protected least significant difference test on Super ANOVA software (Abacus Concepts, Inc., Berkeley, CA).

Results

Exogenous IL-12 administration enhanced morbidity and mortality of Candida albicans-infected mice

The effects of IL-12 administration on C57BL/6 mice challenged with C. albicans were determined using several infection and treatment regimens. Animals were monitored for mortality and median survival time (MST). Three different doses of rmIL-12 (0.001, 0.01, and 0.11 μg/mouse) were given to animals with either a chronic or an acute lethal Candida infection. The results of these studies show that infection was exacerbated by treatment with exogenous IL-12 (Fig. 1, A and B). A significant decrease in MST
(p < 0.05) was observed in both chronically and lethally infected groups treated with all three doses of IL-12 compared with that in their respective saline-treated, infected controls. However, only in the chronic infections were the MST differences significant (p < 0.05) in a dose-dependent fashion.

The enhanced susceptibility to infection imposed by IL-12 was also evident in H&E-stained histologic sections of the kidney, which exhibited tissue necrosis and a lack of cellular infiltration in response to IL-12 treatment of infection (Fig. 2b), compared with infection alone (Fig. 2a). Correspondingly, PAS-stained kidney sections showed a dramatic increase in fungal burden in IL-12-treated samples, indicated by extensive staining of fungal colonies (Fig. 2d). PAS-stained kidney sections from an infected untreated animal showed degraded colonies, indicating a resolving lesion and self-limiting infection (Fig. 2, c and d). Furthermore, we observed a mean increase of about 1 log in recovery of yeast from the organ of clearance, the kidney, as determined by colony-forming units per milligram of tissue in mice treated with IL-12 (0.1 μg/mouse). The log colony-forming unit counts were 3.70 ± 1.08 for the saline-treated mice and 6.55 ± 0.33 for mice that received IL-12 (mean ± SD; n = 8; day 5).

Administration of IL-12 augments IFN-γ and IL-10 secretion, but not IL-4

To correlate the outcome of a chronic Candida challenge with the nature of the immune responses generated in IL-12-treated mice, circulating levels of cytokines were measured by ELISA. In addition, IL-4 production was assayed from in vitro mitogen- and Ag-stimulated supernatant fluids derived from spleen, lymph node, and peritoneal cell populations. The experimental groups of mice were naive, IL-12 treated, Candida infected, and Candida infected plus IL-12. Our data confirm that IL-12 is a potent inducer of
consistent with our IFN-γ knockout data suggest a protective role for endogenous IFN-γ. However, infected, IL-12-treated knockout mice had an MST equivalent to those with infection alone at 13.6 and 13.9 days, respectively. These results suggest that the high level of IFN-γ induced by IL-12 contributed to lethality, while blocking IFN-γ offered protection. Although there appeared to be complete resistance to disease in the infected group receiving anti-IFN-γ alone, the difference in MST was not statistically significant (p > 0.05) compared with that in the groups with infection alone or infection plus a control Ab.

To ensure the complete absence of endogenous IFN-γ during infection, animals deficient in IFN-γ were examined as well. As shown in Figure 6, IFN-γ knockout mice are actually more susceptible to infection than their counterpart wild types, as demonstrated by respective survival rates of 0 and 70% on day 30. This was not the result we initially anticipated based on our in vivo blocking studies. Instead, the IFN-γ knockout data imply that endogenous IFN-γ is needed for resistance to disease in the infected group receiving anti-IFN-γ alone, the difference in MST was not statistically significant (p > 0.05) compared with that in the groups with infection alone or infection plus a control Ab.

To ensure the complete absence of endogenous IFN-γ during infection, animals deficient in IFN-γ were examined as well. As shown in Figure 6, IFN-γ knockout mice are actually more susceptible to infection than their counterpart wild types, as demonstrated by respective survival rates of 0 and 70% on day 30. This was not the result we initially anticipated based on our in vivo blocking studies. Instead, the IFN-γ knockout data imply that endogenous IFN-γ is needed for resistance to disease in the infected group receiving anti-IFN-γ alone, the difference in MST was not statistically significant (p > 0.05) compared with that in the groups with infection alone or infection plus a control Ab.

To ensure the complete absence of endogenous IFN-γ during infection, animals deficient in IFN-γ were examined as well. As shown in Figure 6, IFN-γ knockout mice are actually more susceptible to infection than their counterpart wild types, as demonstrated by respective survival rates of 0 and 70% on day 30. This was not the result we initially anticipated based on our in vivo blocking studies. Instead, the IFN-γ knockout data imply that endogenous IFN-γ is needed for resistance to disease in the infected group receiving anti-IFN-γ alone, the difference in MST was not statistically significant (p > 0.05) compared with that in the groups with infection alone or infection plus a control Ab.
protection, while enhanced IFN-γ production via IL-12 administration can exacerbate Candida infections.

Discussion

The capacity to control disease lies, in part, in the ability of the host to generate an appropriate cell-mediated immune response. More specifically, Th cell interaction with invading micro-organisms influences cytokine networks. Murine candidiasis studies have suggested that resistance is linked to a Th1 response and the production of IFN-γ, while the failure to elicit protective immunity is associated with a Th2 response and the production of IL-4 and IL-10 (6, 14, 24). Accumulating evidence in this and other infectious disease models indicates that IL-12 is crucial for the development of protective Th1 responses and that this cytokine is par-

<table>
<thead>
<tr>
<th>Day</th>
<th>IFN-γ</th>
<th>IL-4</th>
<th>IL-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naive</td>
<td>1</td>
<td>&lt;1.2</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>IL-12</td>
<td>2.9</td>
<td>&lt;1.8</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Candida</td>
<td>28.7</td>
<td>&lt;1.2</td>
<td>0</td>
</tr>
<tr>
<td>Candida + IL-12</td>
<td>45.9</td>
<td>&lt;1.8</td>
<td>0</td>
</tr>
<tr>
<td>IL-12</td>
<td>2</td>
<td>&lt;1.8</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Candida</td>
<td>0.7</td>
<td>&lt;1.8</td>
<td>0</td>
</tr>
<tr>
<td>Candida + IL-12</td>
<td>2.9</td>
<td>&lt;1.8</td>
<td>16.8</td>
</tr>
<tr>
<td>IL-12</td>
<td>3</td>
<td>&lt;1.2</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Candida</td>
<td>1.1</td>
<td>&lt;1.8</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Candida + IL-12</td>
<td>2.9</td>
<td>&lt;1.8</td>
<td>6.7</td>
</tr>
</tbody>
</table>

*Total RNA was extracted from kidneys, snap frozen on days indicated and then analyzed using QC-PCR to measure cytokine message. The table shows number of transcript copies per 1000 copies of the housekeeping gene G3PDH.
particularly potent in its ability to induce the production of IFN-γ, a major mediator of antimicrobial activity (29–32). The present study examined the effects of exogenous IL-12 administration on the progression of disease and the cellular immune response during a chronic systemic infection. Surprisingly, we see that the administration of IL-12 profoundly modified the course and the outcome of infection by adversely affecting the development of protective immunity and exacerbating disease.

Several important observations are relevant to the present results; most strikingly, resistant mice that normally survive at least 3 wk with chronic systemic disease succumbed within a few days when given IL-12 therapy (Fig. 1A). Treatment with IL-12 also accelerated mortality in mice given an acute lethal challenge of C. albicans (Fig. 1B). This notable decrease in host resistance was associated with the absence of an inflammatory response and increased fungal load in the target organs, the kidneys (Fig. 2), as well as with elevated and sustained production of IFN-γ in vivo (Fig. 3).

NO production was correlated with the level of IFN-γ. Initially, it was surprising that this group was also the most susceptible, considering that NO has been implicated in microbial killing (34, 35). Recently, however, it has been suggested that NO is not directly involved in candidacidal activity (33). Instead, NO may still be associated with candidacidal mechanisms that allow other macrophage candidacidal pathways to function (33). This evidence suggests that nitrite/nitrate levels may not accurately predict susceptibility or resistance.

The most dramatic outcome in our studies was that neutralization of IFN-γ in systemically infected animals treated with IL-12 resulted in an enhanced resistance (Fig. 5). Nevertheless, our results obtained from the IFN-γ knockout animal studies are in agreement with those reported by Romani et al. (7) and suggest that IFN-γ does play a role in protection against Candida infections. We hypothesize that overproduction of IFN-γ, mediated through exogenous IL-12 administration, results in enhanced susceptibility to the fungus. This hypothesis is supported by Garner et al. (36), who have shown that administration of IFN-γ during murine candidiasis increased morbidity and mortality as well as yeast colonization in the kidneys. More recently, Qian and Cutler (37), using BALB/c mice that were genetically deficient in IFN-γ demonstrated that this cytokine was not essential for resistance against
disseminated disease. While the reasons for the differences in their data and those of the current study are not completely understood, they may be in part due to the background strain of the mice used (BALB/c vs C57BL/6), the challenge dose given (2.5–5.0 × 10⁶ vs 5.0–13.0 × 10⁵ CFU), and the strain of Candida albicans inoculated (Ca-1 serotype A vs American Type Culture Collection 36082). Nonetheless, their studies do not eliminate IFN-γ having a contributing role in the host response or pathogenesis of this infection.

Previous studies (7, 12–14) have suggested that endogenous production of both IL-12 and IFN-γ may be associated with protective immunity in mice with candidiasis. We now see that IL-12, when administered exogenously, may exert a variety of opposing biologic effects that determine the final outcome of infection. We suggest that IFN-γ and IL-12 may influence the complex interactions that occur between the innate and acquired components of the immune response during this infection. While endogenous IL-12 may be beneficial in promoting protective Th-mediated immunity, exogenous administration of this cytokine and the resultant increase in IFN-γ production may have detrimental effects on the inflammatory response, namely macrophages and neutrophils, which are critical for recovery from candidemia (38, 39). However, to date, no direct effects of IL-12 have been observed on either neutrophil (M. Klemperer, unpublished observations) or macrophage function in vitro. It is possible that IL-12, via IFN-γ, may be affecting the innate immune response by impairing the production of certain cytokines, such as IL-1 (40), which has been shown to protect mice against a lethal C. albicans infection (41, 42), and of chemokines such as IL-8 and JE/MCP-1 (43–45), which are critical for recovery from candidemia (38, 39).

In conclusion, while native IL-12 may be required for the generation of a protective immune response against candidiasis in vivo, the present study shows that its exogenous administration can exacerbate disease by an apparent IFN-γ-dependent mechanism. Understanding how IL-12 is capable of inducing such opposing immunologic effects will be important for our understanding in the utilization of IL-12 and other cytokines in the therapy of infectious diseases in the future.

Acknowledgments

The authors thank Dr. Robert Schaub for his support and helpful discussions during the course of this work and for reviewing the manuscript. We also thank Jana Subramanyam, Tuma Misra, Jamie Erikson, and Sharon Hunter for their technical assistance, and Ron Dattoli for animal care and maintenance.

References


