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IFN-α/β Signaling Is Required for Polarization of Cytokine Responses toward a Protective Type 1 Pattern during Experimental Cryptococcosis

Carmelo Biondo,* Angelina Midiri,* Maria Gambuzza,* Elisabetta Gerace,* Maria Falduto,* Roberta Galbo,* Antonella Bellantoni,* Concetta Beninati,* Giuseppe Teti,* Tomas Leanderson,† and Giuseppe Mancuso1*

The antiviral activities of type I IFNs have long been established. However, comparatively little is known of their role in defenses against nonviral pathogens. We examined here the effects of type I IFNs on host resistance against the model pathogenic yeast Cryptococcus neoformans. After intratracheal or i.v. challenge with this fungus, most mice lacking either the IFN-α/β receptor (IFN-α/βR) or IFN-β died from unrestrained pneumonia and encephalitis, while all wild-type controls survived. The pulmonary immune response of IFN-α/βR−/− mice was characterized by increased expression of IL-4, IL-13, and IL-10, decreased expression of TNF-α, IFN-γ, inducible NO synthetase, and CXCL10, and similar levels of IL-12 mRNA, compared with wild-type controls. Histopathological analysis showed eosinophilic infiltrates in the lungs of IFN-α/βR−/− mice, although this change was less extensive than that observed in similarly infected IFN-γR-deficient animals. Type I IFN responses could not be detected in the lung after intratracheal challenge. However, small, but statistically significant, elevations in IFN-β levels were measured in the supernatants of bone marrow-derived macrophages or dendritic cells infected with C. neoformans. Our data demonstrate that type I IFN signaling is required for polarization of cytokine responses toward a protective type I pattern during cryptococcal infection. The Journal of Immunology, 2008, 181: 566–573.

The single IFN-β and multiple IFN-α subtypes belong to the type I IFN family, whose effects are mediated by activation of a common heterodimeric receptor (IFN-α/βR or IFNAR). Although the antiviral functions of these cytokines have long been clarified (1, 2), more recent studies have highlighted their fundamental role in modulating a variety of innate and adaptive immune responses. Type I IFNs can be considered as primary mediators of host defenses, because they are produced early during infection and regulate hundreds of secondary genes (3, 4). The past few years have witnessed considerable progress in the identification of the microbial stimuli, host cell receptors, and signaling pathways that trigger type I IFN induction. Stimulation with bacterial or viral nucleic acids, as well as LPS from Gram-negative bacteria, results in type I IFN gene induction through distinct, highly ordered processes regulated by multiple transcription factors, especially IFN regulatory factors. These pathways are initiated by the detection of the above-mentioned microbial products by germline-encoded receptors located in the cytosol or membranes of host cells (3, 4). Transmembrane receptors consist of TLR, which are located on the cell surface or in membrane-bound endosomal vesicles. Cytosolic receptors capable of detecting viral nucleic acids include retinoic acid-inducible gene I and melanoma differentiation-associated gene 5. Each of these receptors senses distinct products of viral or bacterial infections and uses distinct transduction pathways to activate type I IFN genes.

The functional activities of IFN-α/β during bacterial infections are complex and incompletely understood. Type I IFN mediates lethal toxicity during endotoxic shock (5) and may have a pathophysiologic role during sepsis (6). Moreover, quite surprisingly, type I IFN signaling was found to be highly detrimental in experimental infections caused by the intracelullar pathogen Listeria monocytogenes (7–9). These effects were associated with attenuated host defenses and apoptosis of immune cells. In contrast, during infection with a variety of common extracellular pathogens, type I IFN signaling was found to play an essential role in host resistance, and to prime macrophages for increased proinflammatory responses (10). Therefore, type I IFN may play different roles during bacterial infection according to the type of pathogen and, possibly, to its lifestyle inside the host (e.g., intracellular vs extracellular).

Despite the increasing incidence of fungal infections and their associated mortality, the presence and possible significance of type I IFN responses in these infections received little attention (11, 12). Antifungal responses involving cytokines other than type I IFNs are becoming elucidated as to the fungal ligands, host cell receptors, and signaling pathways involved. Accumulating evidence indicates that innate recognition of β-glucan by various receptors, including dectin 1, TLR2, and/or the CD11b/CD18 (CR3) integrin, is able to drive inflammatory responses through the production of TNF-α, IL-12, and various chemokines (13–16). Moreover, recognition of mannlike patterns on phospholipomannan and mannanproteins (17) and, possibly, chitin recognition (18), may be involved in immune sensing of fungi.

In the present study, it was of interest to determine whether type I IFN is produced in response to Cryptococcus neoformans and has...
a role in host resistance against this pathogenic yeast. We focused on C. neoformans because it is a model pathogen and a frequent agent of severe meningoencephalitis, particularly in individuals with impaired cell-mediated immunity (19). Protective innate immune responses against cryptococcosis are at least partially dependent on TLR2 and MyD88 signaling (20, 21). Moreover, Th1, as opposed to Th2, responses are essential for clearance of this fungus. In the development of protective Th1 responses, early production of IFN-γ, IL-12, TNF-α, and MIP-1 α is considered to be required (19, 22). In contrast, Th2-type responses, characterized by eosinophil infiltrates and eosinophilic crystal deposition, are likely to play an important pathogenic role (23). Data reported here indicate an essential role of type I IFN signaling in polarization of cytokine responses toward a protective type 1 pattern during cryptococcal infection.

Materials and Methods

Mice

IFN-αR−/− and IFN-αR+/− mice on a 129SvEv background, as well as 129SvEv wild-type (WT)2 mice were purchased from B&K Universal. IFN-β−/− mice on a C57BL/6 background were obtained as described (24), while C57BL/6 WT mice were purchased from Charles River Laboratories. The mice (8-wk old) were housed under pathogen-free conditions in enclosed filter-top cages at the Department of Pathology and Experimental Microbiology (University of Messina, Messina, Italy). All studies were performed in agreement with the European Union guidelines of animal care and were approved by the relevant national committees.

Cryptococcal preparations and reagents

Chemicals were purchased from Sigma-Aldrich, unless indicated otherwise. The highly virulent serotype A strain H99 was purchased from the American Type Culture Collection (no. 208821). For in vivo and in vitro experiments, yeast cells were grown to the exponential phase in a chemically defined medium as previously described (20). Cells were then washed in nonpyrogenic PBS (0.01 M phosphate, 0.15 M NaCl (pH 7.4)), counted on a hemocytometer, and the suspension was adjusted to the desired concentration in PBS. Actual CFU numbers used in each experiment were determined after plating on Sabouraud dextrose agar (Difco Laboratories). With all preparations, endotoxin concentration was found to be <0.1 endotoxin units (EU)/ml. Preliminary experiments showed that at least 20 EU/ml were required to induce IFN-β production in bone marrow-derived macrophage cultures using, as a stimulus, Salmonella enterica se-rovar Minnesota R395 ultrapure LPS (List Biologicals; specific activity 226 EU/pg).

Marine infection models

To induce respiratory infection, WT and knockout mice were anesthetized by the i.p. injection of a mixture of xilazyne (3 mg/kg), tiletamine chlorhydrate (35 mg/kg), and zolazepam chlorhydrate (35 mg/kg). An incision was made through the skin of the ventral neck and 20 μl of a C. neoformans suspension was injected directly into the isolated trachea using a 30-gauge needle. The incision was closed with a sterile wound clip. For i.v. injection, WT and knockout mice received 0.1 ml of a suspension of C. neoformans in the lateral tail vein. Mice were observed daily for signs of disease and lethality. Mice with signs of irreversible disease (e.g., persistent hunching or piloerection, unsteady gait, lethargy) were euthanized. For quantification of fungal burden, organs were homogenized and diluted in 10-fold steps in PBS. Fungal CFUs were determined by plating serially diluted homogenates on Sabouraud dextrose agar.

Bone marrow–derived cells

Bone marrow–derived cells were prepared by flushing femurs and tibiae with sterile RPMI 1640 supplemented with 10% heat-inactivated FCS. The cells were collected by centrifugation and resuspended in hypotonic Tris-ammonium chloride buffer at 37°C for 5 min to lyse RBC (30). After centrifugation, the cells were resuspended in RPMI 1640, supplemented with 5% FCS, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2-ME, as described (30). Medium was supplemented with 100 ng/ml M-CSF and GM-CSF (both obtained from PeproTech) to obtain, respectively, macrophages and conventional dendritic cells (cDC). Every 3 days, half of the medium was removed and substituted with fresh cytokine-supplemented culture medium. To obtain plasmacytoid dendritic cells (pDC), bone marrow cells were grown in IL-3–ligand (100 ng/ml; PeproTech) and pDC were purified by the Plasmacytoid Dendritic Cells Isolation kit (Miltenyi Biotec), as per the manufacturer’s instructions. Cells cultured in M-CSF were found to be, by flow cytometric analysis, >95% positive for CD11b, >85% positive for F4/80, and <5% positive for CD11c. Cells cultured in GM-CSF were found to be >85% positive for CD11c and CD11b and negative for B220. Purity of pDC (CD11c⁺CD11b⁻B220⁻) was found to be >95%. All Abs were purchased from Miltenyi Biotec. Macrophages, cDC and pDC, were stimulated for the

1Abbreviations used in this paper: WT, wild type; EU, endotoxin unit; IP-10, inducible protein 10; ILNOS, inducible NO synthetase; i.e., intratracheal; cDC, conventional dendritic cell; pDC, plasmacytoid dendritic cell.

2 Abbreviations used in this paper: WT, wild type; EU, endotoxin unit; IP-10, inducible protein 10; ILNOS, inducible NO synthetase; i.e., intratracheal; cDC, conventional dendritic cell; pDC, plasmacytoid dendritic cell.
indicated times with live cryptococci at different multiplicities of infection. Poly I:C (50 g/ml; InvivoGen) stimulation was used as a positive control in these experiments.

In vitro restriction of yeast growth

Bone marrow-derived macrophages were obtained from WT and IFN-α/βR−/− mice as described above. Cells (5 × 10^3) with live H99 C. neoformans at a multiplicity of infection of 5:1 (yeast to macrophage ratio) in 96-well flat-bottom microtiter plates. The number of yeasts per well was determined at 0 and 24 h by lysing cells with 0.1% Triton X-100 followed by diluting and spreading onto Sabouraud dextrose agar plates for CFU enumeration, as described (31, 32). Results were expressed as percent anticytotoxic activity according to the formula (1 – (CFU at 24 h/CFU at 0 h)) × 100. Thus, positive percent values indicate killing of C. neoformans.

Cytokine measurements

IFN-β concentration was measured in the supernatants of bone marrow-derived or lung cell cultures by a commercial sandwich enzyme immunoassay using tetramethylbenzidine as the substrate (mouse IFN-β ELISA; PBL Biomedical Laboratories). IFN-γ, TNF-α, IL-13, and IL-4 concentrations were measured in lung cell culture supernatants using the murine IFN-γ-reagent set (Euromine), the mouse TNF-α module set (Bender Med-Systems), the QuantiKine mouse IL-13, and the QuantiKine mouse IL-4 (both obtained from R&D Systems). The lower detection limits of these assays were 15.6 (IFN-β), 25 (IFN-γ), 16 (TNF-α), 1.5 (IL-13), and 2 (IL-4) pg/ml.

Data expression and statistical significance

Cytokine levels and log CFU were expressed as means ± SEM of several determinations, each conducted on a different animal. Differences in cytokine levels and organ CFUs were assessed by one-way ANOVA and the Student-Keuls-Newman test. Survival data were analyzed with Kaplan-Meier survival plots followed by the log-rank test (JMP Software; SAS Institute) on an Apple Macintosh computer. When p values of <0.05 were obtained, differences were considered statistically significant.

Results

IFN-α/βR-deficient mice are hypersusceptible to C. neoformans infection

To investigate the role of type I IFN in host defenses against C. neoformans, we used a murine model of persistent infection mimicking, to some extent, the latency status found in immunocompetent humans (20). In this model, a small dose of cryptococci is administered by the i.t. route, after which the yeasts spread to the lung and other organs without causing overt symptoms of disease for up to 90 days. To assess the role of type I IFN signaling in host resistance, we used IFN-α/βR-deficient mice and WT (129Sv/Ev) controls. Because IFN-γ is known to play a central role in host defenses against C. neoformans, IFN-γR−/− mice were also tested for comparison (33, 34). The animals were infected i.t. with a sublethal dose (1 × 10^3 CFU) of the highly virulent H99 strain and survival was monitored. As shown in Fig. 1A, under
these conditions, all of the WT controls survived while 90 and 50% of, respectively, IFN-γ−/− and IFN-α/βR−/− mice succumbed to infection.

In additional experiments, IFN-α/βR−/− mice and WT controls were infected i.t. and sacrificed at various times after challenge to measure CFUs in the lung and in the brain (Fig. 1, B and C). Fungal burden did not increase in the first 3 days after challenge in either WT or IFN-α/βR−/− mice. In WT mice, lung CFUs were moderately increased at day 7 over values observed at earlier time points, but returned to low levels at day 10 thereafter. In striking contrast, the pulmonary fungal burden of IFNα/βR-deficient mice dramatically increased, starting from day 7, to reach levels that were considerably higher than those observed in WT animals. This increase in lung CFU was paralleled by massive dissemination to the brain. At day 21 postinfection, brain CFU levels were several orders of magnitude higher than those of WT mice.

The above data did not clarify whether, in immune-defective mice, the increased fungal burden in the brain was solely the result of increased dissemination from the lung. To ascertain whether brain defenses were intrinsically impaired in the absence of type I IFN signaling, mice were infected by the i.v. route. Under these conditions, IFN-α/βR−/− mice showed increased lethality and fungal burden in different organs including the brain, liver, and spleen (data not shown). This first set of data indicated that lack of type I IFN signaling resulted in a markedly reduced ability to control fungal growth in different organs.

Previous studies have shown that the severity of infection, as measured by the survival time of WT controls, can profoundly influence the effects of immune deficiencies in a number of different experimental infections, including cryptococcosis (10, 20). For this reason, IFN-α/βR−/− and IFN-γ−/− animals were challenged i.t. with a 50-fold higher dose (5 × 10^5 CFU) than that used in previous studies. Under these conditions, all of the WT animals died within 44 days with a mean survival time of 32.6 days (Fig. 2A). Although there was a tendency for IFN-α/βR−/− to die earlier (mean survival time of 27.1 days), this difference was not statistically significant. Similarly, significant differences in brain and lung CFU were not observed between IFN-α/βR−/− and WT mice using the same high challenge dose (data not shown). Interestingly, under the same conditions, differences in survival between IFN-γR−/− and WT mice were barely significant (p = 0.0499, Fig. 2A), despite the well-documented, profound hypersusceptibility to cryptococcosis of these mice (33). Taken together with previous studies (20), these data suggest that the effects of a number of immune deficiencies, including those studied here, may not be evident under experimental conditions characterized by overwhelming cryptococcosis, with resulting high lethality rates, of control animals.

**Role of IFN-β in C. neoformans infection**

The above data indicated that type I IFNRE is of crucial importance for the ability of the host to restrict *C. neoformans* replication. However, these results did not differentiate between the roles of IFN-α and IFN-β. We therefore investigated the effects of deletion of the IFN-β gene on survival following infection with *C. neoformans*. IFN-β−/− mice were infected i.t. and i.v. with, respectively,
5 × 10² and 2 × 10² organisms. These doses were found, in previous studies, to be sublethal for control WT C57BL/6 mice (our unpublished observations). Using either the i.t. or the i.v. infection route, IFN-β gene-deleted mice were significantly more susceptible to infection than WT mice (Fig. 2, B and C). In addition, brain and lung CFU measured at different time points after i.v. infection were significantly higher in IFN-β⁻/⁻ mice (data not shown). These data indicate that IFN-β has an essential role in host resistance against C. neoformans.

Type I IFN signaling induces polarization toward a type 1 cytokine response

To gain insights as to the mechanisms underlying the increased susceptibility of IFN-α/βR-deficient animals, we measured cytokine mRNAs in the lungs at different times after infection (Fig. 3). No significant elevations over the baseline (i.e., above the values observed in uninfected animals) were observed at day 1 and 2 in either IFN-α/βR⁻/⁻ or WT mice. Interestingly, however, significant elevations in IL-13 levels were observed at day 3 and thereafter in the immune-defective animals, but not in controls. Conversely, in the latter animals, a significant IFN-γ response was detected at day 3. In WT mice, TNF-α mRNA levels were higher at day 7 compared with IFN-α/βR⁻/⁻ mice. Significant IP-10 or iNOS elevations over baseline (uninfected) levels were not detected in the immune-defective animals, although consistent responses were measurable in WT controls (Fig. 3). Relative to WT mice, IL-10 and IL-4 mRNA levels were significantly higher in IFN-α/βR⁻/⁻ mice at different time points. Significant IL-5 mRNA elevations (11.9- ± 2.88-fold over uninfected animals) were observed at 3 wk after infection, but not at earlier time points, in IFN-α/βR⁻/⁻ mice (data not shown). In contrast, no significant IL-5 elevations, relative to uninfected animals, were measured in WT controls at any time point after infection. These data indicate that, in the absence of type I IFN signaling, there is a profound change in the immune response to cryptococcal infection, consisting in decreased type 1 and increased type 2 cytokine mRNA expression.

To ascertain whether variations in cytokine expression were also present at the protein level, lung cells were obtained at 1, 2, and 3 wk after in vivo challenge with C. neoformans and cultured for 24 h. Cytokine concentrations were then measured by ELISA in culture supernatants. Fig. 4 (left panels) shows that, at 1 wk after infection, IFN-γ and TNF-α production was decreased in IFN-α/βR⁻/⁻ cells relative to WT ones. Conversely, IL-13 production was increased at all time points in the defective cells. IL-4 release was also increased in the latter cells at 2 and 3 wk after challenge. Therefore, the ELISA data were in agreement with those obtained by RT-PCR and confirmed the presence of decreased type 1 and increased type 2 responses in the absence of IFN-α/β signaling.

However, these data did not provide clues as to the cell types which were predominantly responsible for the observed alterations in cytokine production. Because T cells are major cytokine producers during cryptococcal infection, we measured cytokine release in lung cells after depletion of T lymphocytes. Fig. 4 shows that T cell depletion did not affect TNF-α and IFN-γ production in either WT or IFN-α/βR-deficient lung cells. Although there was a tendency, at 2 and 3 wk after challenge, toward lower TNF-α and IFN-γ production in T lymphocyte-depleted WT cells, relative to whole T cells, these differences were not statistically significant. However, in the immune-defective cells, T cell removal totally prevented IL-13 and IL-4 release (Fig. 4). These data suggest that non-T cells (likely cells of the innate immunity system) require type 1 IFN signaling in the lung for optimal production of TNF-α and IFN-γ early during infection with C. neoformans. Conversely, T cells appear to be responsible for the increased production of IL-13 and IL-4 observed in the absence of IFN-α/β signaling.

Pulmonary histology of C. neoformans-infected IFN-α/βR⁻/⁻ mice

In further studies, we examined microscopically the lungs of IFN-α/βR⁻/⁻ and IFN-α/βR⁺/⁺ mice at 3 wk after i.t. infection. Results were compared with those observed in IFN-γR⁻/⁻ mice, because the morphological features of C. neoformans infection in these animals have been well-characterized (23, 33). All groups of mice showed inflammatory infiltrates. However, in both IFN-α/βR⁻/⁻ and IFN-γR⁻/⁻ mice, these infiltrates were more extensive than in WT animals (Fig. 5). In addition, numerous cryptococcomas with giant fungal forms were observed in the lungs of both groups of immune-deficient mice while rare cryptococci were found in WT mice (Fig. 5). Eosinophils were evidenced by phenol red staining in tissue section of...
either IFN-α/βR−/− or IFN-γR−/− mice, but not in control C. neoformans-infected WT animals. However, the eosinophilic infiltrate was more extensive in IFN-γR−/− than in IFN-α/βR−/− mice (Fig. 5). Thus, the lungs of C. neoformans-infected IFN-α/βR−/− mice showed morphological features that were qualitatively similar to those of IFN-γR−/− animals. Such features, including a predominantly eosinophilic infiltrate, have been repeatedly associated with a type 2 cytokine pattern.

Induction of IFN-β by cryptococcal stimulation

The above data indicated a crucial role of type I IFN signaling in cryptococcal infection. However, it is unknown whether fungi, including C. neoformans, are capable of inducing type I IFN responses. To clarify this point, we first examined the lung homogenates obtained in the experiments summarized in Fig. 3 for the presence of IFN-β and IFN-α4 mRNA. However, no differences in either IFN-β or IFN-α4 expression were found between infected and uninfected lungs at any time points after challenge (data not shown). Similarly, we could not detect IFN-β in the supernatants of cultured lungs obtained at different times after infection (data not shown). Because macrophages and DC are key elements of innate immune responses, we examined these cell types in vitro for their ability to up-regulate IFN-α/β genes in response to C. neoformans.

To this end, we infected bone marrow-derived in vitro-differentiated macrophages, cDC, and pDC with various doses of C. neoformans strain H99. Poly I:C, a well-characterized IFN-α/β inducer, was used as a positive control. Fig. 6 shows that, but consistent, IFN-β elevations were detected by ELISA in supernatants of C. neoformans-infected bone marrow-derived DC and macrophages. Such responses were detectable only in a very narrow dose range (i.e., at a multiplicity of infection ranging from 5:1 to 8:1). At higher cryptococcal doses, there was abrogation of IFN-β release, concomitantly with morphological evidence, in phagocytes, of cell pathology, including increased turbidity, rounding, and detachment. No response could be measured in pDC (data not shown). We next measured IFN-β and IFN-α4 mRNA levels in macrophages at various times after stimulation. IFN-β elevations were first detected at 4 h after stimulation, peaking at 6 h, and going back at baseline values at 12 h. Only minor elevations were detected in IFN-α4 mRNA levels. These data demonstrate that macrophages and cDC, but not pDC, are able to produce moderate IFN-β levels in response to C. neoformans infection.

Virus-induced IFN-α/β can act autocrinoously in producing a cell-autonomous state of resistance against viral replication. Therefore, it was of interest to ascertain whether C. neoformans-induced IFN-β production in macrophages has a role in their ability to restrict intracellular fungal growth. To this end, we infected cultures of bone marrow-derived macrophages obtained from IFN-β-deficient mice and from WT controls. Anticytotoxic activity was assessed by measuring CFU in macrophage cultures at 0 and 24 h after the addition of yeasts. Percent anticytotoxic activity was 31 ± 4.3 and 29 ± 5.7 in macrophages from WT and IFN-β-deficient mice. These differences were not statistically significant, indicating that IFN-β responses have no role in the cell-autonomous ability of macrophages to restrict in vitro cryptococcal growth.
Discussion
Control of cryptococcal infection depends on the development of adaptive responses involving both CD8+ and CD4+ T cells (34). Early production of type 1 cytokines, including IL-12, TNF-α, and IFN-γ, by cells of the innate immune system is considered essential to polarize adaptive responses toward a protective Th1 pattern (35). For example, transient neutralization of TNF-α during the first week after challenge resulted in permanently impaired long-term clearance of pulmonary C. neoformans infection and in increased type 2 cytokine levels (36). A host-defense pattern involving cross-regulating protective type 1 and detrimental type 2 cytokine responses is observed not only in cryptococcosis, but also in the vast majority of infections caused by intracellular microbes, including the model bacterial pathogen L. monocytogenes (37).

During listeriosis, type I IFN signaling has a marked detrimental effect, resulting in a decreased ability of the host to control bacterial growth (7–9). Quite opposite effects were observed here using a cryptococcosis model, because it was found that the IFN-α/β receptor and IFN-β are required for effective host defenses. The effect of IFN-α/βR deficiency was marked, with a phenotype (consisting of increased fungal burden, eosinophilic lung infiltrates, and type 2 cytokine production) that was qualitatively similar to that of IFN-γ deficiency. Our data raise the possibility that IFN-α/β may be a fundamental factor for host defenses against not only viruses and common bacteria (10), but also fungi. It is possible that L. monocytogenes is unique among microbial pathogens for its ability to exploit an IFN-α/β-dependent pathway to promote pathogenesis, although further studies involving different infectious agents are needed to prove this hypothesis.

To gain insights about the mechanisms underlying the protective effects of type I IFN signaling in pulmonary cryptococcosis, we measured the kinetics of fungal burden and cytokine responses in IFN-α/βR−/− mice and WT controls. Fungal burden was similar in the two groups of mice during the first 3 days after challenge. At day 3, we measured, in WT animals, a significant response involving IFN-γ, but not IL-13 or other cytokines. Conversely, IL-13, but not IFN-γ, was induced at day 3 in IFN-α/βR−/− mice. After these early events, the curves describing fungal burden started to diverge in the two groups of mice. Despite increased fungal burden, relative to WT animals, decreased IFN-γ, TNF-α, and iNOS, expression was measured in IFN-α/βR−/− mice at 7 days or later times after challenge.

Therefore, the inability of IFN-α/βR−/− mice to control cryptococcal infection can be explained by decreased expression of a number of known host defense factors, including iNOS, IFN-γ, and TNF-α. iNOS can be induced by either IFN-α/β or IFN-γ and is a key enzyme for the production of NO, which, in turn, is required for killing of C. neoformans by macrophages (38). Decreased IFN-γ expression may also explain the hypersusceptibility to infection of IFN-α/βR−/− mice, given the fundamental role of this cytokine in many aspects of anticytrococcal immunity, including Th1 polarization (34, 35). Moreover, our data are consistent with the known ability of IFN-α/β signaling to up-regulate IFN-γ production in various cell types of the innate and adaptive immunity system, including dendritic cells (39), macrophages (10), and T cells (40). Because early production of TNF-α likely plays a major role in clearance of cryptococcal pulmonary infection (36), decreased TNF-α expression may also have contributed to the phenotype of IFN-α/βR−/− mice. It is likely that cells of the innate immune system were mainly responsible for TNF-α and IFN-γ release in our model, because the production of these cytokines occurred early after infection and was not affected by T cell depletion. Moreover, it has been shown that NK and NKT cells produce IFN-γ early during cryptococcal infection (41, 42). It will be of interest to more precisely identify in our model the cell types that require IFN-α/β signaling for optimal production of TNF-α and IFN-γ during experimental cryptococcosis. Taken together with previous studies (10), our data suggest that, in the context of infection, IFN-α/β signaling induces a priming state in cells of the innate immune system, leading to increased production of proinflammatory cytokines. Interestingly, in the present study, the only type 1 cytokine to be expressed at similar levels in IFN-α/βR−/− mice and controls was IL-12. It is well-established that IFN-α/β signaling can result in decreased IL-12 production, which is, in turn, up-regulated by IFN-γ (3, 43). Therefore, because our IFN-α/βR−/− mice had decreased IFN-γ expression, the opposing effects of absent type I and reduced type II IFN signaling may have balanced each other in these animals, leading to unchanged IL-12 mRNA levels.

Consistent with decreased TNF-α and IFN-γ induction, we found, in IFN-α/βR-deficient mice, increased expression of type 2 cytokines, such as IL-13, IL-4, and IL-5, and of the regulatory cytokine IL-10. Increased IL-13 expression in these animals was particularly prominent, because it occurred early (during the first week) after infection and steadily increased thereafter, while an IL-13 response was totally absent in WT animals. Cell depletion experiments indicated that T lymphocytes were the predominant cell type responsible for IL-13 production. This data points to an important role of type I IFN signaling in down-regulating, either directly or indirectly (e.g., through IFN-γ), IL-13 expression by T cells. Studies are underway to better clarify the mechanism underlying this effect.

The present study, as well as previous ones (10, 20), emphasizes the importance of using different challenge doses, including sublethal ones, in assessing the effects of various host factors on susceptibility to infection. The major effects of type I IFN might have been missed in the present study using only high doses of C. neoformans (e.g., doses producing 100% lethality in WT controls). Under high-dose conditions, the protective effects of type I IFN may have been overshadowed by pathophysiologic phenomena associated with excessive release of this cytokine, as observed during severe sepsis (6) or endotoxin shock (5). In addition, the massive release of glucuronoxylomannan, a known immunsuppressive factor (44), might have obscured the effects of IFN-α/β signaling during overwhelming cryptococcosis. At any rate, it is seems reasonable to use, for this kind of study, low-dose challenges, which are more likely to mimic the vast majority of naturally occurring situations, including interactions of C. neoformans with the human host.

We showed here that C. neoformans can induce IFN-β gene expression in cells of the innate immunity system, including macrophages and cDC. However, it should be noted that IFN-β induction was low in extent and detectable only in a narrow range of cryptococcal doses. Moreover, it cannot be discerned from our studies how much of the effects of type I IFN signaling is due to constitutive vs induced IFN-α/β production during cryptococcal infection. It should be noted, in this respect, that constitutive IFN-α/β production is known to be required for some cell functions, including efficient IFN-γ signaling and high-level IFN-α/β production in response to viral stimulation (3).

Type I IFN induction depends on the engagement of specific innate immunity “sensors,” including TLRs and cytosolic receptors, leading to activation of IFN regulatory factors. We previously reported that TLR2 has an important role in anticytrococcal host defenses (20). However, neither TLR2 complexes (TLR2/TLR6 and TLR2/TLR6) nor dectin-1, which is known to cooperate with TLR2 in recognition of fungal β-glucan (45), is considered capable of IFN-α/β induction. Theoretically, TLR4 engagement by cryptococcal components, including glucuronoxylomannan (44) may
lead to IFN-α/β secretion, as in the case of LPS-induced TLR4 stimulation. However, it is unlikely that TLR-4 is required for C. neoformans-induced IFN-β release, because TLR4 deficiency, unlike IFN-α/β deficiency, failed to result in hypersusceptibility to infection (20). Therefore, it will be of interest to ascertain whether specific cryptococcal products can trigger IFN-α/β release.

In conclusion, our data indicate that type I IFN is a crucial host defense factor against the model pathogenic yeast C. neoformans. We show that IFN-α/β signaling primes host cells for decreased type 2 and increased type 1 cytokine responses. Our data support the possibility that type I IFN plays a fundamental role in host defenses against fungal, in addition to viral, pathogens.

Disclosures

The authors have no financial conflict of interest.

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