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Immunological basis for susceptibility and resistance to pulmonary blastomycosis in mouse strains

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Abstract

The immunological basis for a >10-fold resistance of outbred CD-1 mice compared to inbred BALB/c mice to pulmonary blastomycosis was investigated. Bronchoalveolar macrophages (BAM) from CD-1 mice killed yeast cells of *Blastomyces dermatitidis* (Bd) by 25% and this increased to 59% when activated by IFN-γ. In contrast, BAM from BALB/c mice lacked significant killing (5%) of Bd but could be activated by IFN-γ for enhanced killing (19%). Peritoneal macrophages (PM) from CD-1 mice had significant fungicidal activity for Bd (43%) and this increased to 63% with IFN-γ treatment. By contrast, PM from BALB/c mice did not significantly kill Bd (14%) but were activated by IFN-γ for significant killing (24%). Fungicidal activity of peripheral blood polymorphonuclear neutrophils (PMN) from CD-1 (87%) was greater than that of BALB/c (75%) ($P < 0.05$). Macrophage inflammatory protein-1α (MIP-1α) production by BAM from BALB/c was significantly less than that from CD-1 in response to co-culture with Bd. IFN-γ production by CD-1 spleen cells in response to concanavalin A (Con A, 1 μg/ml) was 8-fold greater than that by BALB/c spleen cells. In contrast, BAM and PM from BALB/c mice in co-culture with Bd secreted several-fold more TNFα than BAM or PM from CD-1 mice. IL-2 production by BALB/c spleen cells in response to Con A was 3- to 4-fold greater than that by CD-1 spleen cells. Depressed IL-2 production by Con A stimulated CD-1 spleen cells correlated with depressed proliferative responses. Resistance of CD-1 mice to pulmonary blastomycosis correlates with enhanced fungicidal activity of BAM, PM, PMN, and IFN-γ production by Con A stimulated spleen cells, compared to BALB/c mice. Consistent with the in vitro enhancement of effector cell function by IFN-γ, in vivo therapy with IFN-γ significantly ($P < 0.0001$) improved survival of BALB/c mice with pulmonary blastomycosis.

Keywords: Blastomycosis; Susceptibility; Mouse strains; Cytokines; Immunomodulation

1. Introduction

Previous studies showed that susceptibility to pulmonary blastomycosis varied in different inbred strains of mice [1]. C3H/HeJ were the most susceptible, DBA/1J the most resistant, and BALB/c mice had intermediate resistance when challenged intranasally with yeast form *Blastomyces dermatitidis* (Bd). Mitogen-induced proliferation responses of normal lymph node cells (LNC) from susceptible C3H/HeJ mice were 2-fold greater than the responses of LNC from resistant DBA/1J mice. Susceptibility of C3H/HeJ mice correlated with 100-fold higher antigen-specific antibody levels at 48 days post-infection compared to infected resistant DBA/1J mice,

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which in retrospect suggests a Th-2 type of response to infection. These findings left unresolved a definitive immunological basis for resistance vs. susceptibility to pulmonary blastomycosis.

In the present study we have reinvestigated the immunological basis for differences in susceptibility between mouse strains. We found that CD-1 outbred mice are 10-fold more resistant than BALB/c to pulmonary blastomycosis. If there are inherent differences between strains, the differences would affect the course of infection, and then there would be differences in immune responsiveness that are secondary to the progression of infection. For this reason we investigated possible immunological differences in uninfected mice. To find correlates of resistance and susceptibility we measured IFN-γ, IL-2, and IL-10 production by spleen cells to a T-cell mitogen concanavalin A (Con A). Fungicidal activity of bronchoalveolar macrophages (BAM), peritoneal macrophages (PM), and peripheral blood polymorphonuclear neutrophils (PMN) against Bd was determined. Moreover, the capacity of these phagocytes to be activated for enhanced fungicidal activity by IFN-γ was also assessed. Proinflammatory cytokine (tumor necrosis factor, TNFα) and chemokine (macrophage inflammatory protein-1α, MIP-1α) productions by BAM or PM co-cultured with Bd were also determined. The possible basis for resistance of CD-1 mice vs. BALB/c mice to pulmonary blastomycosis is herein reported.

2. Methods and materials

2.1. Mice

Outbred male CD-1 mice were obtained from Charles River Lab., Hollister, CA and inbred BALB/c mice from Simonsen Lab., Gilroy, CA. Age-matched groups of mice, 6–12 weeks old, were used as cell sources for the in vitro studies or the in vivo challenge studies. Mice were pathogen-free, housed with sterilized cages and bedding, supplied with sterilized chow, and acidified water.

2.2. Blastomyces dermatitidis

Bd (ATCC 26199) from storage under water at 4 °C was transformed to the yeast phase by passage on blood agar plates (BAP) at 37 °C. The isolate was passed on BAP for in vivo and in vitro studies in the same fashion, i.e. 3–4 times, before each study. Yeast phase growth from BAP was harvested into saline, washed once in saline, serially diluted in saline, and counted in a hemocytometer.

2.3. Pulmonary infection

Bd yeast cell inocula were prepared in saline. Mice were infected by intranasal (i.n.) instillation of a 30 μl inoculum under light anesthesia. Under these conditions the swallow reflex is suppressed and the inoculum is aspirated into the lungs [2]. Mortality differences between groups are best demonstrated when there is $<100\%$ and $>0\%$ mortality so inoculum sizes producing extreme results are avoided. Based on preliminary data, groups of CD-1 mice (10 mice per group) received inocula of 3000 or 7500 colony forming units (cfu) of Bd to produce some lethality. Groups of BALB/c mice (10 mice per group) were infected i.n. with inocula of 300, 120, or 60 cfu of Bd. Mice were observed daily and mortality recorded. Autopsy confirmed pulmonary blastomycosis.

In other experiment groups of 10 BALB/c mice were infected i.n. with 432 cfu of Bd. Mice were injected s.c. with 0.1 ml of saline (control), IFN-γ 20,000 U, or IFN-γ 40,000 U on days 0, 1, and 2 after infection.

2.4. Bronchoalveolar macrophages (BAM)

Lungs of mice were lavaged with phosphate buffered saline containing 0.1% ethylene-diamine-tetra-acetic acid (EDTA) and 10% (v/v) fetal bovine serum (FBS) adjusted to pH 7.2 [3]. Cells were obtained by repeated 0.5 ml lavages of lungs using a total 10 ml lavage fluid. Cells were pelleted by centrifugation (200g, 10 min). Pelleted cells from 5 to 10 mice were pooled, washed once with RPMI-1640 (Sigma Chemical Co., St. Louis, MO). Cells were treated with 0.85% NH_4Cl to lyse any contaminating red blood cells, washed once with RPMI-1640, suspended in RPMI-1640 + 10% FBS (v/v) + penicillin (100 U/ml) and streptomycin (100 μg/ml) (CTCM), and counted in a hemocytometer. Cells were suspended in 1×10^6/ml CTCM and dispensed 0.1 ml/well of microtest plate (Costar A/2, #3696, Corning, NY). After 2 h at 37 °C in 5% CO_2 + 95% air, nonadherent cells were aspirated, each monolayer rinsed once with 0.2 ml of RPMI-1640. Microscopic examination verified that monolayers consisted of BAM as previously reported [3]. To assess fungicidal activity BAM were cultured overnight with or without IFN-γ, supernatants removed, and then challenged with 0.1 ml of Bd yeast cells in CTCM + 10% (v/v) fresh mouse serum for 2 h at 37 °C in 5% CO_2 + 95% air. Cultures were harvested by aspiration of well contents followed by several washes with distilled water resulting in a total volume of 5 ml. Microscopic examination of harvested material indicated that there was no clumping of yeast cells. Harvested material was plated on BAP, incubated at 35 °C for 4 days, colony counts made, and cfu per culture calculated. Fungicidal activity was defined as reduction of inoculum cfu by the formula: % killing = (1 − [experimental cfu/inoculum cfu]) × 100.

For generation of supernatants from BAM + Bd cultures, microtest plates (Corning #3596) were used. Cells (0.2 ml, 1×10^6/ml CTCM) were plated per well and adherent cells (BAM) were challenged with Bd