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In Vivo Activation of Peripheral Blood Polymorphonuclear Neutrophils by Gamma Interferon Results in Enhanced Fungal Killing

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The effect of in vivo administration of murine recombinant gamma interferon (IFN) on the fungicidal activity of murine peripheral blood polymorphonuclear neutrophils (PB-PMNs) was studied. Mice were injected intramuscularly with 250, 2,500, 25,000 or 250,000 U of IFN 5 h before collection of peripheral blood. Purified PB-PMNs were cocultured in vitro with *Blastomyces dermatitidis* yeast cells for 2 h. PB-PMNs from untreated mice killed 44.5 ± 12.5% of the fungal inoculum, whereas PB-PMNs from mice treated with 25,000 or 250,000 U of IFN showed significantly enhanced in vitro killing (68.0 ± 9.4% [$P < 0.005$] and 72.3 ± 1.1% [$P < 0.001$], respectively). Treatment with 250 or 2,500 U of IFN or 25,000 U of heated (100°C, 15 min) IFN had no effect. The IFN-induced activation of PB-PMNs was transitory. Significant enhancement of PB-PMN killing activity occurred 1, 2, or 5 h after in vivo IFN administration, but no enhancement was observed 16 or 24 h after IFN treatment. Enhanced fungicidal activity by PB-PMNs from mice treated for 5 h with 25,000 U of IFN correlated with an increased release of superoxide anion (O_2^-) in vitro after stimulation of PB-PMNs with phorbol ester; normal PB-PMNs and IFN-activated PB-PMNs, respectively, produced 2.2 ± 2.5 and 23.5 ± 4.8 nmol of O_2^- per 10^6 PB-PMNs per 30 min ($P < 0.005$). The exogenous addition of compounds that antagonize or inhibit the formation of oxygen radicals (superoxide dismutase, catalase, dimethyl sulfoxide, or sodium azide) significantly inhibited fungal killing by both normal and IFN-activated PB-PMNs. In addition to the enhanced microbicidal activity and superoxide generation demonstrated in vitro with constant cell numbers, there was a transient leukocytosis (particularly neutrophilia) in peripheral blood at doses of IFN and at times after IFN administration where enhanced activity was also demonstrated. In summary, our results indicate that PB-PMNs can be activated in vivo for enhanced killing of a fungal target. The enhanced killing capacity of IFN-activated PB-PMNs is due at least in part to the enhancement of oxidative killing mechanisms.

Blastomyces dermatitidis is a soil-borne, thermally dimorphic fungus endemic to the United States (15, 21). Inhalation of fungal particles can result in an initial pulmonary infection that may be self-limited (33) or that may disseminate to other tissues such as the skin or bone (25, 35). The mechanisms for containment of infection to the lungs and for the resolution of disease are not clearly understood. In the murine model (19, 40), the contribution of intact cell-mediated immunity is evident, since resistance to pulmonary challenge can be achieved by passive transfer of sensitized lymphoid cells but not by immune serum (7). A role in the containment of blastomycosis by alveolar macrophages and polymorphonuclear neutrophils (PMNs) is suggested since, histopathologically, these cells are found in large numbers in the well-circumscribed lesions of the lungs (5, 19, 40).

It is documented that murine PMNs can be activated in vivo through the mediation of an immune reaction to kill fungal targets (12, 28). PMNs elicited intraperitoneally in immune mice by injection of nonviable *B. dermatitidis* yeast cells (12) or PMNs from the peripheral blood of these mice (27) significantly reduced inoculum CFU of fungi during coculture. Soluble mediators that may be in part responsible for this effect include lymphokines such as gamma interferon (IFN), since IFN has been demonstrated to activate peritoneal PMNs (26) and peripheral blood PMNs (PB-PMNs) (C. J. Morrison and D. A. Stevens, Int. J. Immunopharmacol., in press) for fungicidal activity in vitro.

We therefore examined the effect of in vivo administration of IFN on the capacity of murine PB-PMNs to kill *B. dermatitidis* in vitro. We report here that intramuscularly administered IFN can activate PMNs for enhanced fungal killing in vitro. To our knowledge, this is the first report of activation of PB-PMNs for enhanced fungicidal activity by in vivo administration of IFN. Enhanced killing is shown to be mediated in part by an increased production of oxygen metabolites by IFN-activated PMNs. IFN may therefore have a therapeutic role in host defense against fungal pathogens.

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MATERIALS AND METHODS

Animals. Specific-pathogen-free BALB/cByJIMR male mice (Institute for Medical Research, San Jose, Calif.) 8 to 10 weeks of age were used.

Fungi. Inocula of *B. dermatitidis* ATCC 26199, a strain demonstrated to be virulent in these mice (5), were prepared for in vitro challenge by harvest of 72-h cultures from blood agar plates (BAP; BBL Microbiology Systems, Cockeysville, Md.) incubated at 37°C. Fungal cells were washed, and dilutions were plated in quadruplicate on BAP to determine the number of CFU per milliliter.

Reagents and media. Tissue culture medium (TCM) consisted of RPMI 1640 medium supplemented with 10% (vol/

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vol) heat-inactivated fetal bovine serum and 100 U of penicillin plus 100 μg of streptomycin per ml (GIBCO Laboratories, Grand Island, N.Y.). Murine IFN produced by recombinant DNA technology was supplied by Genentech, Inc., South San Francisco, Calif. (39). Superoxide dismutase (SOD), catalase, dimethyl sulfoxide (DMSO), sodium azide, ferricytochrome *c*, phorbol 12-myristate 13-acetate, Histopaque 1077, and dextran (molecular weight, 250,000) were purchased from Sigma Chemical Co., St. Louis, Mo. Dulbecco phosphate-buffered saline was purchased from GIBCO.

Activation of PB-PMNs in vivo. Each mouse received 0, 250, 2,500, 25,000, or 250,000 U of IFN (0.1 ml per mouse in sterile phosphate-buffered saline) intramuscularly 5 h before collection of peripheral blood cells. In time-course studies, each mouse received 0 or 25,000 U of IFN intramuscularly at 1, 2, 5, 16, or 24 h before PB-PMN harvest.

PB-PMNs. Peripheral blood was obtained by axillary bleeding of mice. PB-PMNs and mononuclear cells were purified from whole blood by Ficoll-Hypaque centrifugation followed by dextran sedimentation as previously described (27). Cells were counted in a hemacytometer. Differential counts of purified PB-PMN and mononuclear cell preparations from control (n = 10) and IFN-treated (all doses) (n = 23) animals were the same. The PB-PMN preparations were 87.1 ± 2.6% PMN, 9.1 ± 2.4% lymphocytes, and 3.8 ± 0.8% monocytes in controls and 87.9 ± 2.8, 8.5 ± 2.5, and 3.6 ± 1.1%, respectively, in IFN-treated animals. The mononuclear cell preparations were 3.6 ± 1.8, 93.8 ± 2.7, and 2.6 ± 1.1%, respectively, in controls and 3.6 ± 1.5, 93.8 ± 2.3, and 2.6 ± 1.0%, respectively, in IFN-treated animals.

Cocultures of PB-PMNs and *B. dermatitidis*. PB-PMNs (5×10^5 in 0.1 ml of TCM) were challenged with 10^3 CFU of *B. dermatitidis* in 0.1 ml of TCM plus 0.02 ml of fresh normal mouse serum per test plate well (Micro Test III plates; Becton Dickinson Labware, Oxnard, Calif.). In selected experiments, test wells containing PB-PMNs received 0.02 ml of SOD, catalase, DMSO, or sodium azide immediately before the addition of *B. dermatitidis*. The inhibitors, at concentrations shown to abrogate PMN fungal killing, did not affect PMN viability as determined by trypan blue exclusion. Cocultures were incubated for 2 h at 37°C in 5% CO_2–95% air and harvested with distilled water as previously described (27). Dilutions were made in saline, and portions were plated on blood agar. The number of CFU per well was determined by counting colonies formed on BAP after 5 days of incubation at 37°C. Mononuclear cell preparations were studied in the same effector/target cell ratio and other conditions as described for PB-PMNs.

Determination of superoxide production by PB-PMNs. Superoxide anion production was quantitated in a ferricytochrome *c* reduction assay with phorbol 12-myristate 13-acetate as the stimulus. PB-PMNs (0.5×10^6 to 1×10^6 per test tube) were incubated for 30 min at 37°C in a shaking water bath with ferricytochrome *c* (40 μM), phorbol 12-myristate 13-acetate (2 μg/ml), and fresh normal mouse serum (2 to 4%). The final volume in each tube was 2.5 ml. Selected tubes contained SOD (6,000 U/ml); subtraction of values for these tubes from those for test samples gave the SOD-inhibitable reduction of ferricytochrome *c*. After incubation at 37°C, test tubes were placed on ice and then centrifuged at 1,000 × *g* at 4°C for 10 min. The supernatants were read spectrophotometrically at 550 nm. Ferricytochrome *c* reduction was determined by using an extinction coefficient of $2.1 \times 10^4\ M^{-1}\ cm^{-1}$.

Statistical analyses. The Student *t* test was used for comparisons between groups. Means are given as plus or minus the standard deviation. *P* values of <0.05 were considered significant.

TABLE 1. Effect of in vivo IFN treatment on in vitro killing of *B. dermatitidis* by PB-PMNs

Treatment[a]	Reduction of 0-h inoculum, % ± SD[b] (n)	P[c]
No PMNs	3.6 ± 4.9 (11)	
PB-PMNs		
IFN (0 U)	44.5 ± 12.5 (7)	
IFN (250 U)	49.0 ± 9.0 (1)	NS
IFN (2,500 U)	53.0 ± 8.5 (2)	NS
IFN (25,000 U)	68.0 ± 9.4 (6)	<0.005
IFN (250,000 U)	72.3 ± 1.1 (2)	<0.001

[a] Mice were injected intramuscularly with IFN 5 h before peripheral blood collection. PB-PMNs were purified as described in Materials and Methods.

[b] Mean percent reduction in inoculum CFU for the number of experiments within parenthesis or, for a single experiment, for the mean of quadruplicate wells.

[c] Comparison of inoculum reduction by PB-PMNs from untreated versus treated mice by Student's *t* test; NS, not significantly different by this test.

RESULTS

Effect of in vivo IFN administration on in vitro killing of *B. dermatitidis* by peripheral blood leukocytes. Mice were injected intramuscularly with 0 to 250,000 U of murine IFN 5 h before the collection of peripheral blood. Purified PB-PMNs and mononuclear cell fractions were challenged in vitro with *B. dermatitidis* yeast cells (Table 1). PB-PMNs from mice treated with 25,000 or 250,000 U of IFN showed significantly enhanced fungal killing ($P < 0.005$ and $P < 0.001$, respectively) compared with PB-PMNs from untreated animals. Treatment of mice with phosphate-buffered saline (diluent) or with 250 or 2,500 U of IFN had no effect (Table 1). Heat treatment (100°C, 15 min) of IFN (25,000 U) before injection resulted in the loss of PB-PMN-activating capacity: the percent reduction of inoculum CFU by IFN-activated PB-PMNs versus PB-PMNs from mice given heat-treated IFN was 73.7 ± 7.4% versus 56.4 ± 3.6%, respectively ($P < 0.01$; quadruplicate samples).

Peripheral blood mononuclear cells were unable to kill *B. dermatitidis* in vitro whether mononuclear cells were from untreated or IFN-treated mice. Results were as follows: for untreated mice, 7.8 ± 7.1% reduction of inoculum CFU, n = 7; with 250 U of IFN, 5.4 ± 7.6%, n = 2; with 2,500 U of IFN, 1.0 ± 1.4%, n = 2; with 25,000 U of IFN, 6.7 ± 7.4%, n = 5; and with 250,000 U of IFN, 4.1 ± 5.7%, n = 2 ($P > 0.05$).

Time course for in vivo activation of PB-PMNs by IFN; IFN effect on cell counts. Mice were either untreated or injected intramuscularly with 25,000 U of IFN at 1, 2, 5, 16, or 24 h before collection of peripheral blood. Purified PB-PMNs and mononuclear cells were tested for their ability to kill *B. dermatitidis* in vitro. PB-PMNs from mice treated with IFN 1, 2, or 5 h before cell harvest demonstrated significantly enhanced fungicidal ability relative to control PB-PMNs from untreated mice (Table 2). The IFN-induced PB-PMN-activating effect was transient, however, since at 16 or 24 h after IFN administration PB-PMNs were no longer activated (Table 2).

The peripheral blood mononuclear cells were not affected by in vivo administration of IFN regardless of the time interval tested. The percent reduction of inoculum was as

TABLE 2. Time course for in vivo activation of PB-PMNs by IFN

Treatment interval[a] (h)	Reduction of 0-h inoculum, % ± SD[b] (n)	% Increase in kill over control[c]	P[d]
No treatment	47.1 ± 11.8 (10)		
1	81.8 ± 5.8 (3)	74	<0.001
2	63.2 ± 4.5 (2)	34	<0.01
5	68.0 ± 9.4 (6)	44	<0.005
16	51.7 ± 6.9 (1)	10	NS
24	49.0 ± 7.3 (3)	4	NS

[a] Mice were either untreated or injected intramuscularly with 25,000 U of IFN at the designated time before collection of peripheral blood.
[b] See Table 1, footnote *b*.
[c] Percent increase in killing by PB-PMNs from IFN-treated mice relative to PB-PMNs from untreated mice.
[d] See Table 1, footnote *c*.

follows: no IFN, 10.9 ± 7.1%, $n = 12$; 1 h after IFN, 10.3 ± 10.1%, $n = 3$; 2 h after IFN, 12.8 ± 18.0%, $n = 2$; 5 h after IFN, 6.7 ± 7.4%, $n = 5$; 16 h after IFN, 13.9 ± 12.7%, $n = 2$; and 24 h after IFN, 13.9 ± 13.2%, $n = 3$.

Although the differential counts of the PB-PMN and mononuclear cell preparations were, as already verified in Materials and Methods, identical in control and IFN-treated mice, the total cells in both preparations were altered by IFN treatment. This reflected a transient leukocytosis in IFN-treated (but not heat-treated IFN-dosed) mice, largely due to a transient neutrophilia. Cell counts in control mice included $5.9 \times 10^5 \pm 1.7 \times 10^5$ PMN, $1.5 \times 10^6 \pm 0.7 \times 10^6$ lymphocytes, and $6.2 \times 10^4 \pm 2.1 \times 10^4$ monocytes per ml of blood ($n = 17$). After administration of 25,000 U of IFN, the PMNs in blood increased 185, 305, and 185% at 1, 2, and 5 h ($P < 0.025$, all three times, $n = 4$ to 8) but were not significantly different than those in controls at 16 and 24 h. Monocytes were significantly increased at 1 and 2 h (119 and 157%, respectively; $P < 0.05$ for both; $n = 4$) but not at later times, and lymphocytes were not significantly increased. This effect was studied for dose responsiveness in detail at 5 h after IFN injection. The increase in PMNs was significant for 250,000 U of IFN (220%; $P < 0.025$) as well as 25,000 U but was not significantly different for 250 or 2,500 U ($n = 2$ to 8). None of these doses produced a significant increase in monocytes or lymphocytes. Heat-treated IFN (25,000 U) did not produce a significant increase in any of the three cell types.

Comparison of superoxide anion release by normal versus IFN-activated PB-PMNs. Release of active oxygen metabolites has been correlated with enhanced killing of microbial targets by PMNs (1, 13, 16, 28). One such active oxygen metabolite demonstrated to be involved in fungal killing is the superoxide anion (O_2^-) (35). We measured the quantity of O_2^- released in vitro by PB-PMNs of normal and IFN-treated (25,000 U intramuscularly 5 h before peripheral blood collection) mice to determine whether enhanced fungal killing by activated PB-PMNs could be due to an increased production of O_2^- or related oxygen metabolites.

Normal PB-PMNs released 2.2 ± 2.5 nmol of SOD-inhibitable O_2^- per 10^6 PB-PMNs per 30 min ($n = 3$), whereas IFN-activated PB-PMNs released over 10 times more O_2^- (23.5 ± 4.8 nmol; $n = 3$; $P < 0.005$).

Purified peripheral blood mononuclear cells tested in this system did not produce detectable levels of superoxide anion in three experiments.

Effect of inhibitors of oxygen metabolism or metabolites on in vitro killing by normal or IFN-activated PB-PMNs. When inhibitors of toxic oxygen metabolites and their production were added exogenously to culture wells immediately before fungal challenge of PB-PMNs, significant inhibition of fungal killing was observed. SOD, an antagonist of superoxide anion, inhibited killing by 81% ($P < 0.01$) to 85% ($P < 0.001$) for normal and IFN (25,000 U)-activated PB-PMNs, respectively (Table 3). Catalase, which breaks down H_2O_2 to water and oxygen, reduced killing by 54% ($P < 0.005$, normal PB-PMNs) to 64% ($P < 0.001$, IFN-activated PB-PMNs). DMSO (a hydroxyl radical scavenger) and sodium azide (a singlet oxygen antagonist and myeloperoxidase inhibitor) also reduced killing significantly (DMSO, 50% [$P < 0.01$] and 89% [$P < 0.001$]; azide, 96% [$P < 0.001$] and 100% [$P < 0.001$], respectively, for normal and IFN-activated PB-PMNs) (Table 3).

TABLE 3. Effect of antagonists of oxygen metabolism and/or metabolites on in vitro killing by normal or IFN-activated PB-PMNs

PB-PMN type[a]	Inhibitor added[b]	Reduction of 0-h inoculum, % ± SD[c] (n)	% Inhibition of kill[d]	P[e]
Normal	None	44.5 ± 12.5 (7)		
	SOD (6,000 U/ml)	8.5 ± 12.0 (2)	81	<0.01
	Catalase (11,000 U/ml)	20.6 ± 3.7 (2)	54	<0.005
	DMSO (300 mM)	22.3 ± 4.3 (2)	50	<0.01
	Azide (1.0 mM)	1.7 ± 1.5 (2)	96	<0.001
IFN activated	None	68.0 ± 9.4 (6)		
	SOD (6,000 U/ml)	10.2 ± 5.5 (2)	85	<0.001
	Catalase (11,000 U/ml)	24.3 ± 8.8 (2)	64	<0.001
	DMSO (300 mM)	7.7 ± 11.1 (1)	89	<0.001
	Azide (1.0 mM)	0.0 ± 4.0 (1)	100	<0.001

[a] PB-PMNs were obtained from the peripheral blood of untreated mice (normal PB-PMNs) or mice treated intramuscularly with 25,000 U of IFN 5 h before collection of peripheral blood (IFN-activated PB-PMNs).
[b] Inhibitors were added exogenously to culture wells immediately before challenge of PB-PMNs with *B. dermatitidis*.
[c] See Table 1, footnote *b*.
[d] Percent inhibition of fungal killing in the presence, compared to with that in the absence, of inhibitors of oxygen metabolism or metabolites.
[e] Comparison of inoculum reduction in the presence of versus in the absence of inhibitors of oxygen metabolism or metabolites.

The addition of SOD to cultures of PB-PMNs from mice treated in vivo with a higher dose of IFN (250,000 U) also abrogated the killing activity of these cells (killing in the absence of SOD, 71.5%; killing in the presence of SOD, 0%).

DISCUSSION

This is the first report demonstrating that the in vivo administration of IFN can activate PB-PMNs for enhanced in vitro killing of fungi. We should emphasize here that since the differential counts of cell preparations of control and IFN-treated mice were the same, the observations of enhanced killing (as well as O_2^- production) cannot be attributed to IFN-induced changes in the differential of the preparations but rather to IFN-induced changes in the killing power and O_2^- production of the cells studied. Activation occurred in a dose-dependent manner, with maximal enhancing effects observed at the highest dose tested (250,000 U). Higher doses of IFN were not tested bacause of limited availability of the lymphokine. However, it would be of interest to examine higher doses of IFN to see whether further augmentation of fungicidal activity could be

achieved. Previous experience with the in vitro activation of peritoneal PMNs and of macrophages indicates that higher doses of IFN are not necessarily more effective than lower doses; that is, a maximum threshold is reached where higher concentrations (100,000 U/ml) are less stimulatory than lower ones (10,000 U/ml) (8, 26).

The observed in vivo activation of PB-PMNs was transitory. Significant PB-PMN-enhancing effects occurred 1, 2, or 5 h after IFN treatment, but no enhancement of in vitro fungicidal activity was observed 16 or 24 h after IFN administration. Maximal enhancement of fungicidal activity occurred 1 h after in vivo administration of IFN. It is not known whether an exposure interval of less than 1 h would result in significant activation of PB-PMNs. However, our data indicate that PB-PMNs can be activated in vivo on a time course consistent with that observed for the in vitro activation of peritoneal PMNs (9, 26) or PB-PMNs (Morrison and Stevens, in press); that is, PMNs can be activated for enhanced fungal killing after in vitro exposure to lymphokines (9, 26; Morrison and Stevens, in press) for 1 h. Shorter in vitro exposure (30 min) did not fully activate peritoneal PMNs (26).

The transitory nature of the IFN-induced activation of PB-PMNs may be due to the short half-life (38) of IFN itself or to the turnover of circulating PMNs, which, once activated, may be short-lived. The 10-fold higher release of superoxide anion from IFN-activated PB-PMNs not only may contribute to an enhanced fungicidal capacity but may also provide a self-destructive environment (37) for PB-PMNs.

The activation would be magnified in vivo by the transient neutrophilia; not only was the fungicidal activity enhanced with the PMN/target cell ratio constant (Table 1), but also there were more PMNs in the peripheral blood at the times (1, 2, and 5 h) when fungicidal activity was increased. These would be additive effects in the enhancement of PMN fungicidal power in peripheral blood. The peak in neutrophilia occurred later (2 h) than the peak in fungicidal activity reflected in the in vitro conditions with cell numbers constant (1 h; Table 2). The enhanced fungicidal activity could be explained by IFN causing an enrichment of PMNs in peripheral blood, with PMNs having enhanced microbicidal activity from other compartments, or by independent IFN effects of mobilizing PMNs and enhancing microbicidal activity in some or all of the PMN population.

Additional evidence for the contribution of oxygen-dependent mechanisms to the killing of *B. dermatitidis* by PB-PMNs lies in the significant reduction of fungal killing by these cells upon exogenous addition of antagonists of toxic oxygen metabolites and their production (SOD, catalase, DMSO, sodium azide) to the coculture wells. The various actions of these antagonists, also discussed elsewhere (1, 28), suggest that superoxide anion, hydrogen peroxide, hydroxyl radical, singlet oxygen, and hypochlorous acid may all have important roles in the killing. These results complement those demonstrating a role for oxidative processes in fungal killing by immunologically activated peritoneal PMNs (13, 28). In contrast, pulmonary macrophages have been reported to utilize nonoxidative fungicidal mechanisms to kill *B. dermatitidis* (11) or the pulmonary fungal pathogens *Paracoccidioides brasiliensis* (6) and *Coccidioides immitis* (36). Nonoxidative mechanisms have also been shown to contribute to fungal killing by PMNs (22).

Lipopolysaccharide (such as may be hypothesized to be present as a contaminant in IFN preparations), which may enhance or depress PMN functions, cannot reasonably explain our findings. Lipopolysaccharide is heat stable, and IFN is not; heating preparations to inactivate IFN but not lipopolysaccharide removed the capacity to activate PMNs. Moreover, these IFN preparations have been purified to undetectable amounts by the *Limulus* cell lysate assay for lipopolysaccharide (26; Morrison and Stevens, in press). Finally, lipopolysaccharide does not activate PMNs in vitro for enhanced fungicidal activity (26; Morrison and Stevens, in press).

Peripheral blood mononuclear cells did not kill *B. dermatitidis* and were not activated to do so after in vivo administration of any one of a broad range of IFN concentrations (250 to 250,000 U), including those shown to activate PB-PMNs for enhanced fungicidal activity. In vivo administration of 25,000 U of IFN 1, 2, 5, 16, or 24 h before collection of mononuclear cells also did not result in fungicidal activity by these cells. Earlier studies (26; Morrison and Stevens, in press) showed that removal of PMNs from PMN preparations (treated in vitro with IFN) with monoclonal anti-PMN antibodies plus complement removed IFN-induced anti-*Blastomyces* cellular activity, also indicating lack of involvement of mononuclear cells in killing by PMN preparations. This is not to suggest that purified peripheral blood monocytes, and especially macrophages derived from mononuclear cells in the peripheral blood, are not capable of being activated for fungicidal activity given the appropriate conditions. Indeed, there is strong evidence that pulmonary macrophages can be activated by in vitro or in vivo exposure to IFN for enhanced killing of fungal pathogens such as *C. immitis* (2), *P. brasiliensis* (6), and *B. dermatitidis* (6, 10). Peripheral blood mononuclear cells therefore differ from macrophages in that the former are not activated by IFN to kill *B. dermatitidis* in vitro (Morrison and Stevens, in press) or in vivo. Indeed, in in vitro activation experiments where mononuclear cells were studied in an effector/target cell ratio 10 times higher than that used with PMNs failed to demonstrate killing (Morrison and Stevens, in press).

The mediation of host resistance through the action of soluble lymphokines on phagocytic cells is consistent with our observations of the enhancement of fungal killing by PB-PMNs obtained from mice immunized by subcutaneous injection of viable *B. dermatitidis* cells and subsequently challenged intraperitoneally with *B. dermatitidis* antigen (27). That a local (peritoneal) immune reaction exerts effects on cells of the peripheral blood suggests the action of a soluble mediator such as IFN and/or other lymphokines or cytokines. Lymphokines have been suggested to play an important role in host defense against disease via the activation of PMNs or macrophages for enhanced bactericidal (14, 31), protozoacidal (4, 18, 30), and fungicidal (3, 8, 26, 41) activity. Further evidence lies in the observation that in vivo administration of IFN results in the protection of experimental animals from infections with *Toxoplasma gondii* (24) or *Listeria monocytogenes* (20), whereas the in vivo administration of anti-IFN antibody exacerbated toxoplasmosis (38) or *Rickettsia conorii* (23) infection in mice.

We conclude that in vivo administration of IFN results in the activation of PB-PMNs for enhanced killing of *B. dermatitidis* in vitro. We suggest that IFN may have a therapeutic role in fungal infections. Since in vivo administration of IFN has previously been shown to enhance macrophage fungicidal activity (6), the therapeutic advantages could be multiplied. A role for IFN may be particularly appropriate where normal functions of the PMNs and macrophages are impaired. The impaired production of lymphokines and IFN has been implicated in the predisposition of some groups of

compromised hosts to opportunistic infections, including those caused by fungi (17, 29, 32). The administration of IFN to such patients may therefore result in the activation of PMNs and macrophages and thus potentially enhance host defense capabilities in vivo.

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