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Enhancement of Susceptibility of CB-17 Mice to Systemic Candidiasis by Poly(I · C)-Induced Interferon

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Poly(I · C) enhanced the susceptibility of CB-17 (BALB/c) mice to acute systemic candidiasis. Poly(I · C), supernatants from poly(I · C)-treated macrophages, or alpha and beta interferons suppressed macrophage candidacidal activity in vitro. Thus, poly(I · C)-induced interferons may enhance the susceptibility of CB-17 mice to candidiasis by suppressing macrophage candidacidal activity in an autocrine fashion.

The immunomodulatory effects of several different cytokines on candidacidal activity of macrophages (Mϕ) have been demonstrated (1, 2, 6, 8, 10, 13, 14). Gamma interferon (IFN-γ), interleukin-1, lipopolysaccharide, and granulocyte-macrophage colony-stimulating factor can enhance the candidacidal activity of human alveolar Mϕ and peripheral blood monocytes (13, 14). In mice, colony-stimulating factor 1 enhances the candidacidal activity of exudate peritoneal Mϕ in vitro (10), whereas IFN-γ enhances the candidacidal activity of resident peritoneal Mϕ in vitro (2). Tumor necrosis factor, interleukin-1, and IFN-γ have been shown to increase the candidacidal activity of a murine Mϕ cell line (1). The effect of IFN-γ in vivo may depend on the immune status of the animal (6). Recently, we reported (8) that Mϕ from poly(I · C)-treated SCID mice showed decreased candidacidal activity in vitro and that in vitro treatment of Mϕ with poly(I · C) suppressed Mϕ candidacidal activity. The suppressive effect of poly(I · C) on Mϕ candidacidal activity was overcome by antibody to IFN-α/β (8), suggesting that poly(I · C)-induced IFNs suppressed the candidacidal capacity of Mϕ. In this study, we examined the effect of poly(I · C) and purified murine IFN-α and IFN-β on the candidacidal activity of Mϕ in immunocompetent CB-17 mice.

These studies were done as described previously (8). Briefly, mice were injected with poly(I · C) (100 μg, intraperitoneally; 0.1 ml) immediately prior to intravenous (i.v.) challenge with 10^4 CFU (0.1 ml) of *Candida albicans*. In experiments with *C. albicans*-preinoculated mice, the mice were inoculated with *C. albicans* by i.v. injection of 10^4 (0.1 ml) live *C. albicans* and 10^4 (0.1 ml) formalin-killed *C. albicans* cells at 2 and 1 week, respectively, prior to initiation of poly(I · C) treatment and challenge with *C. albicans*. In experiments with antibody to IFN, mice were inoculated intraperitoneally with 200 μg (0.5 ml) of anti-IFN-γ (hybridoma R4-6A2, ATCC HB 170; prepared as described previously [8]), 10^4 neutralizing units (0.5 ml) of anti-IFN-α/β (sheep polyclonal antibody; National Institute of Allergy and Infectious Diseases, Bethesda, Md.), or a mixture of both antibodies (total volume, 0.5 ml) 24 h before and again immediately prior to injection with poly(I · C). Some mice were also injected i.v. with 200 μg (0.2 ml) of rabbit anti-asialo GM1 (Wako Chemicals, Dallas, Tex.) 3 days prior to injection with poly(I · C) and *C. albicans*. Control mice received the equivalent volumes of saline. The number (CFU) of viable *C. albicans* in internal organs was determined by plating homogenates of organs on Sabouraud dextrose agar plates. Preparation and purification of antibodies and assays for splenic natural killer (NK) cell activity were done as described previously (8). The effects of poly(I · C), purified IFN, and antibody to IFN on the in vitro candidacidal activity of thioglycolate (TG)-elicited peritoneal Mϕ were determined by incubating Mϕ for 18 h with the specific reagent. Mϕ monolayers were then washed, *C. albicans* was added to give an effector/target cell ratio of 10:1, and Mϕ candidacidal activity was determined in a 4-h CFU reduction assay (8). TG-elicited peritoneal exudates contained >90% Mϕ by differential staining (Diff-Quik stain; American Scientific Products, McGaw Park, Ill.). The concentrations of reagents used were as follows: poly(I · C), 100 μg/ml; anti-IFN-α/β, 6,000 neutralizing units/ml; anti-IFN-β (Lee Biomolecular, San Diego, Calif.), 5.6×10^4 neutralizing units/ml; purified murine IFN-α (National Institute of Allergy and Infectious Diseases), 1,000 U/ml; purified murine IFN-β (National Institute of Allergy and Infectious Diseases), 1,000 U/ml.

Treatment with poly(I · C) in vivo (100 μg, intraperitoneally) enhanced the susceptibility of CB-17 mice to subsequent i.v. challenge with *C. albicans*, as evidenced by a 5- to 100-fold increase in the number of CFU isolated from the livers, spleens, and kidneys of poly(I · C)-treated mice 24 h after challenge compared with saline control mice (Table 1). Similar, but less significant, increases in CFU were seen in the brain (data not shown). Poly(I · C)-enhanced susceptibility of CB-17 mice to acute systemic candidiasis was abrogated by concurrent treatment with antibody to both IFN-α/β and IFN-γ. The number of CFU isolated from the livers, spleens, and kidneys of mice treated with both antibodies plus poly(I · C) was significantly lower than that recovered from poly(I · C)-treated mice and comparable to the number of CFU isolated from the livers, spleens, and kidneys of saline control mice (Table 1). Treatment with antibody to either IFN-α/β or IFN-γ only partially abrogated the effect of poly(I · C), as shown by the intermediate numbers of CFU in the livers and kidneys of these mice compared with poly(I · C)-treated mice and saline control mice (Table 1). These data suggest that poly(I · C)-enhanced susceptibility to acute systemic candidiasis is mediated by IFNs. Since poly(I · C) induces in vivo production of IFN-γ by NK cells (4) and that of IFN-α and IFN-β by Mϕ (5, 9), we also examined the effect of poly(I · C) in CB-17 mice depleted of

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TABLE 1. Poly(I · C)-induced IFN enhances the susceptibility of CB-17 mice to acute systemic candidiasis

Group[a]	Log_{10} CFU/g (dry wt) of tissue ± SEM in given organ		
	Liver	Spleen	Kidney
Saline + *C. albicans*	2.79 ± 0.16	2.98 ± 0.08	3.63 ± 0.18
Poly(I · C) + *C. albicans*	3.24 ± 0.13[b]	3.25 ± 0.17[b]	5.43 ± 0.07[b]
Anti-IFN-γ + poly(I · C) + *C. albicans*	3.21 ± 0.03[b]	2.97 ± 0.03[c]	4.88 ± 0.06[b,c]
Anti-IFN-α/β + poly(I · C) + *C. albicans*	3.14 ± 0.01[b]	2.89 ± 0.05[c]	4.93 ± 0.04[b,c]
Anti-IFN-γ + anti-IFN-α/β + poly(I · C) + *C. albicans*	3.00 ± 0.11[c]	2.83 ± 0.04[c]	3.85 ± 0.06[c]
Anti-asialo GM1 + poly(I · C) + *C. albicans*	3.11 ± 0.09[b]	3.21 ± 0.08[b]	5.05 ± 0.05[b]
Anti-asialo GM1 + anti-IFN-α/β + poly(I · C) + *C. albicans*	2.89 ± 0.02[c]	2.86 ± 0.06[c]	4.04 ± 0.40[c]

[a] Mice were injected with saline, poly(I · C) (100 μg, intraperitoneally), or poly(I · C) plus antibody and challenged i.v. with 10^4 CFU of *C. albicans*. Mice were sacrificed 24 h after challenge, and the number of CFU in the internal organs was determined. Each group includes data from four to eight mice from two to four different experiments.

[b] Significantly different ($P < 0.05$) from saline controls challenged with *C. albicans* alone by Student's *t* test.

[c] Significantly different ($P < 0.05$) from poly(I · C)-treated, *C. albicans*-infected mice by Student's *t* test.

NK cell activity with anti-asialo GM1 (Table 1). In NK cell-depleted mice, poly(I · C)-enhanced susceptibility to candidiasis was abrogated by concurrent treatment with antibody to IFN-α/β alone. The number of CFU in the livers, spleens, and kidneys of NK cell-depleted mice treated with anti-IFN-α/β plus poly(I · C) was significantly lower than that recovered from NK cell-depleted mice treated with poly(I · C) and comparable to that recovered from saline control mice, suggesting that poly(I · C)-enhanced susceptibility to acute systemic candidiasis is mediated in part by IFN-α/β derived, most likely, from Mϕ. Splenic NK cell activity was virtually undetectable (<3%) in mice treated with anti-asialo GM1, as determined by a standard 4-h ^{51}Cr-release assay with YAC-1 target cells (8; data not shown). Poly(I · C) also enhanced the susceptibility of *C. albicans*-preinoculated CB-17 mice to acute systemic candidiasis (data not shown).

The effect of poly(I · C) in vitro on Mϕ candidacidal activity was examined. Incubation with poly(I · C) (100 μg/ml) for 18 h in vitro significantly reduced the candidacidal capacity of exudate (TG-elicited) peritoneal Mϕ (Table 2). The mean candidacidal activity of poly(I · C)-treated Mϕ was 6.7%, while medium control cultures showed 21.6% killing. The suppressive effect of poly(I · C) was abrogated by the concurrent addition of antibody to IFN-α/β, as evidenced by the 25.7% killing obtained with these cultures (Table 2).

These data suggest that poly(I · C)-induced suppression of Mϕ candidacidal activity is mediated by IFN-α/β. Therefore, we examined the effect of purified murine IFN-α and IFN-β on the candidacidal activity of Mϕ. As shown in Table 2, treatment for 18 h with either IFN-α (1,000 U/ml) or IFN-β (1,000 U/ml) significantly reduced the candidacidal activity of Mϕ to 1.7 and 1.5%, respectively (Table 2). Reduced Mϕ candidacidal activity was also found with IFN-α or IFN-β at 100 U/ml, but not with either at 10 U/ml (data not shown). The suppressive effect of either cytokine was abrogated by the concurrent addition of antibody to IFN-α/β or IFN-β, as shown by the percent killing (19.0 and 23.5%, respectively) obtained with these treatments (Table 2). Incubation with poly(I · C), IFN, or antibody to IFN did not affect the viability of Mϕ by trypan blue staining. Antibody to IFN did not significantly affect Mϕ candidacidal activity.

To determine whether IFN-α and IFN-β act in an autocrine manner to suppress Mϕ candidacidal activity, we examined the effect of antibody to IFN-α and IFN-β on suppression of candidacidal activity induced by supernatants from poly(I · C)-treated Mϕ. As shown in Table 2, treating Mϕ for 18 h with supernatants harvested from poly(I · C)-treated Mϕ significantly reduces their candidacidal capacity (2.3% killing) compared with that of medium control cultures (19% killing). Concurrent addition of antibody to IFN-α/β or IFN-β, however, abrogated the supernatant-induced suppression, as shown by the 16.3 and 22.3% killing obtained with these cultures (Table 2).

The data in this study are in agreement with our previous report (8) that IFNs induced by poly(I · C) suppress Mϕ candidacidal activity. In addition, we have confirmed that purified IFN-α or IFN-β can suppress Mϕ candidacidal activity and that these cytokines act in an autocrine manner. The exact mechanisms by which IFN-α and IFN-β suppress Mϕ candidacidal activity is unclear at present. However, previous studies have demonstrated that IFN-α/β can inhibit colony-stimulating factor 1-induced monocytopoiesis (11) and suppress colony-stimulating factor 1-induced expression of mannose receptors on peritoneal exudate Mϕ (10). Thus,

TABLE 2. Effect of poly(I · C) and IFN-α/β on candidacidal activity of peritoneal exudate Mϕ

Mϕ treatment[a]	Mϕ candidacidal activity in vitro (mean % killing ± SEM)[b]
Control (media only)	21.6 ± 3.5
Poly(I · C) (100 μg/ml)	6.7 ± 2.3[c]
Poly(I · C) + anti-IFN-α/β (6,000 U/ml)	25.7 ± 4.4
IFN-α (1,000 U/ml)	1.7 ± 1.0[c]
IFN-β (1,000 U/ml)	1.5 ± 0.9[c]
IFN-α + anti-IFN-α/β	19.0 ± 1.7
IFN-β + anti-IFN-β	23.5 ± 2.7
Anti-IFN-α/β	20.1 ± 2.3
Anti-IFN-β	25.8 ± 1.9
Supernatant[d]	2.3 ± 1.0[c]
Supernatant + anti-IFN-α/β[d]	16.3 ± 2.2
Supernatant + anti-IFN-β[d]	22.3 ± 1.0

[a] TG-elicited peritoneal Mϕ were incubated with poly(I · C), IFN, or anti-IFN for 18 h and then tested for candidacidal activity in a 4-h CFU assay. Data are the results of three experiments with TG-elicited Mϕ from two mice in each experiment. Standard errors are for total number (six) of mice used.

[b] At an effector/target cell ratio of 10:1.

[c] Significantly different ($P < 0.05$) from medium controls by Student's *t* test.

[d] TG-elicited peritoneal Mϕ were incubated for 18 h with supernatant from poly(I · C)-treated Mϕ, or supernatant and anti-IFN, and then tested for candidacidal activity in a 4-h CFU assay.

the suppressed candidacidal activity of Mϕ observed in the present study could reflect impaired phagocytosis of yeast-phase *C. albicans*. Other studies have shown that IFN enhances killing of *Listeria monocytogenes* by nonoxidative killing mechanisms (12), and both IFN-α and IFN-β have been shown to suppress production of superoxide anions by murine Mϕ (7). Since oxidative mechanisms are thought to be important for Mϕ candidacidal activity (2, 3), conceivably, the suppressed candidacidal activity of Mϕ treated with IFN-α/β could result from suppressed intracellular killing of yeast-phase *C. albicans*.

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