INFECTION AND IMMUNITY, Jan. 1990, p. 260–262
0019-9567/90/010260-03$02.00/0

Vol. 58, No. 1

Prostaglandin E_2 Enhances and Gamma Interferon Inhibits Germ Tube Formation in *Candida albicans*

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Received 17 July 1989/Accepted 11 October 1989

Prostaglandin E_2 (PGE_2), an immunosuppressive monokine that increases intracellular cyclic AMP (cAMP) levels, stimulated *Candida albicans* germ tube formation. Dibutyryl cAMP (dB-cAMP) and isoproterenol, other compounds that increase cAMP levels, also stimulated germination. Gamma interferon (IFN-γ), a product of cellular immune system activation, inhibited *Candida* germ tube formation, even in the presence of PGE_2, dB-cAMP, and isoproterenol. Thus, PGE_2 and IFN-γ as well as having opposing roles in the suppression or activation of cell-mediated immunity, are also antagonists for the yeast-to-hyphal transition of *C. albicans*.

Candida albicans is an opportunistic dimorphic fungus which induces clinical symptoms of vaginitis in susceptible women. Hormonal alterations accompanying pregnancy or endocrinopathies, treatment with immunosuppressive medications, or changes in the vaginal microflora after antibiotic usage enhance vaginal infections by this organism (18). Some women with candidal vaginitis also exhibit a deficiency in their cell-mediated immune response to *C. albicans* (22). This appears to be related to a localized vaginal allergic response to *C. albicans*, components of semen, or other allergens (23). Vaginal secretions from these women contain prostaglandin E_2 (PGE_2) (23), a potent immunosuppressor (11).

In this communication, we show that products of suppressed or activated lymphoid cells also affect the yeast-to-hyphal transition of this organism. This will influence pathogenicity, since the hyphal form of *C. albicans* is associated with increased virulence (20, 21).

C. albicans 925 was used in all the experiments. Yeast cultures were grown on Sabouraud agar slants overnight at 28°C, in a 5% CO_2 humidified atmosphere.

Germ tube formation was measured in glucose beef extract (GBE) broth, a medium used for the presumptive identification of *C. albicans* (Flow Laboratories, McLean, Va). Yeast cells were harvested from a 24-h culture, suspended (10^8/ml) in 0.1 ml of GBE medium (pH 7.0), and mixed with an equal volume of GBE broth containing the cytokine or other compound to be tested. Incubation was for 120 min at 37°C. Samples (25 μl) were removed in quadruplicate, 400 cells were counted under 200× power, and the mean numbers of budding yeast forms and germ tube forms were determined. Replicate aliquots differed in the extent of germination by less than 10%. Under these conditions of time, temperature, and cell concentration, 50% of the yeast forms reproducibly underwent germination in the absence of added compounds.

PGE_2 and $PGF_{2\alpha}$ (Biomol Research Labs, Plymouth Meeting, Pa.) were dissolved in 95% ethanol and diluted in GBE medium to the desired concentration, between 20 pg and 10 ng. Alpha interferon (IFN-α) and gamma interferon (IFN-γ) (Interferon Science Inc., New Brunswick, N.J.) were added to GBE medium in concentrations between 4 and 100 U. Dibutryl cyclic AMP (dB-cAMP) and isoproterenol (Sigma Chemical Co., St. Louis, Mo.) were examined at concentrations between 10^{-5} and 10^{-7} M. Higher concentrations of isoproterenol were toxic to the yeast cells. Isoproterenol was dissolved in 95% ethanol and diluted in medium to yield final ethanol concentrations of <0.1%. dB-cAMP was dissolved in phosphate-buffered saline and diluted in GBE medium. Controls of GBE broth containing between 0.1 and 0.001% ethanol were always incubated in parallel to the test samples. At these concentrations, ethanol had no effect on germ tube formation.

To further assess the effect of IFN-γ on germination, IFN-γ (40 U) in GBE medium was incubated with or without a 1:100 dilution of immunoglobulin G mouse monoclonal antibody to either human IFN-γ (Interferon Science) or herpesvirus (control) (Serotec, Blackthorn, England). After 60 min at 37°C, an equal volume of *C. albicans* in GBE medium was added and germination was determined as described above.

The significance of altered germination rates in the presence of added compounds was evaluated by chi-square analysis, using the Yates correction factor.

Germination was enhanced by PGE_2 at concentrations above 100 pg; greater than 75% stimulation was observed at concentrations of 500 pg and higher. In contrast, $PGF_{2\alpha}$ was without effect on germination over the 20-pg to 10-ng range tested (Fig. 1).

In contrast to the stimulatory effect of PGE_2, IFN-γ inhibited germination. Maximal inhibition (35 to 50%) was obtained in the presence of 40 U of IFN-γ. A second interferon, IFN-α, was not inhibitory to *C. albicans* germ tube formation at comparable concentrations (Fig. 2A). The capacity of IFN-γ to inhibit germination was obviated in the presence of monoclonal antibody to IFN-γ (data not shown). To eliminate the possible involvement of endotoxin contamination in the inhibition of germination, the experiments were repeated in the presence of 3 μg of polymyxin B sulfate per ml, an endotoxin inhibitor. Quantitatively similar results were obtained (data not shown).

The effect of IFN-γ on the stimulation of *C. albicans* germination by PGE_2 was also examined. In the presence of 30 ng of PGE_2, *Candida* germination was progressively

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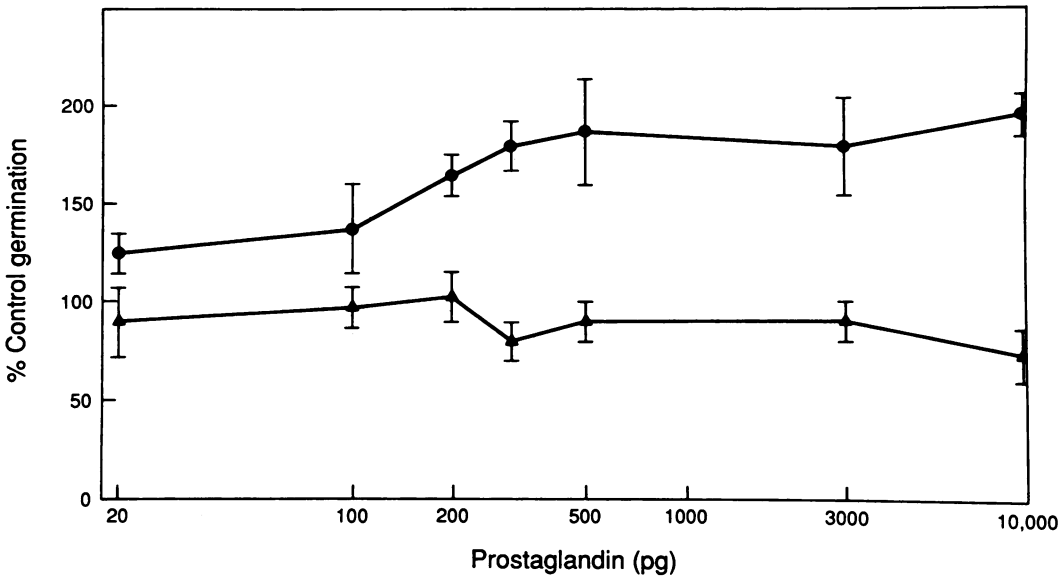

FIG. 1. Effects of prostaglandins PGE_2 and $PGF_{2\alpha}$ on *C. albicans* germ tube formation. *C. albicans* yeast forms (10^8 cells per ml) were incubated in GBE medium with and without PGE_2 (●) or $PGF_{2\alpha}$ (▲). After 120 min, aliquots were removed for determination of the number of yeast and germinating forms present. The vertical lines indicate the standard deviations from three separate experiments.

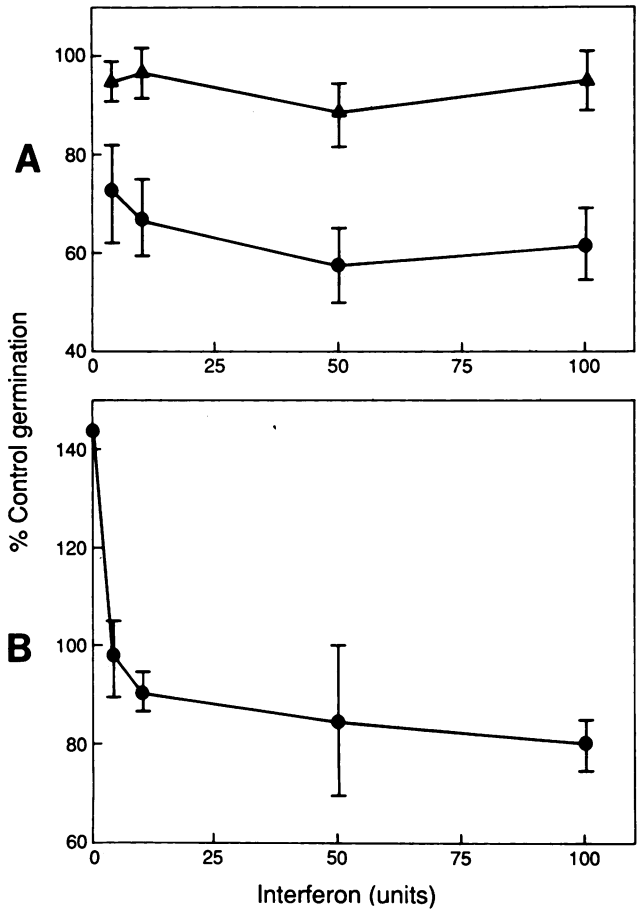

FIG. 2. Effects of IFN-γ and IFN-α on *C. albicans* germ tube formation. (A) *C. albicans* yeast forms (10^8 cells per ml) were incubated for 120 min in GBE medium with no additions or with IFN-γ (●) or IFN-α (▲). The numbers of yeast cells that initiated germ tube formation compared with the controls during this time were measured. The vertical lines indicate the standard deviations from three separate experiments. (B) *C. albicans* yeast forms were incubated in GBE medium containing 30 ng of PGE_2 and various concentrations of IFN-γ. The numbers of cells undergoing the yeast-to-germ tube transmission were measured for each culture. The vertical lines indicate the standard deviations from two separate experiments.

inhibited by increasing levels of IFN-γ over a 4- to 100-U range (Fig. 2B).

PGE_2 exerts its immunosuppressive effect by increasing the intracellular concentration of cAMP in lymphocytes and macrophages (5, 10). To examine whether this mechanism might also be involved in PGE_2-stimulated germ tube formation, we tested whether other compounds that increase intracellular cAMP levels could also promote germination of *C. albicans*. Both isoproterenol, a β-adrenergic catecholamine, and dB-cAMP, an analog of cAMP, stimulated *Candida* germ tube formation (Table 1). Isoproterenol increasingly enhanced germ tube formation over the 10^{-7} to 10^{-5} M range tested ($P < 0.01$), while dB-cAMP significantly enhanced germination at concentrations of 10^{-6} M ($P < 0.025$) and 10^{-5} M ($P < 0.01$). Similar to its effect on PGE_2-mediated germination, IFN-γ also inhibited germ tube induction by isoproterenol and dB-cAMP (data not shown).

Germ tube formation in *C. albicans* can also be induced by changes in pH (6), temperature (2), and nutrient content of the medium (9). In the present experiments, the pH of the medium was measured after the addition of all test compounds and at the termination of each protocol. In all cases, the pH remained unaltered at 7.0. The experimental design also negated possible influences of temperature shifts or nutrients on germination.

Germ tube formation in *C. albicans* has previously been shown to occur subsequent to an increase in cAMP, while cyclic GMP levels remained unchanged (17). In the present study, three compounds that increase cAMP levels, PGE_2, isoproterenol and dB-cAMP, stimulated the yeast-to-hyphal

TABLE 1. Effect of isoproterenol and dB-cAMP on *Candida* germ tube formation[a]

Concn (M)	Mean % control germination (SD)	
	Isoproterenol	dB-cAMP
10^{-7}	126.0 (7.6)	111.8 (13.0)
10^{-6}	138.4 (1.9)	135.5 (8.3)
10^{-5}	151.7 (3.5)	118.5 (2.6)

[a] *C. albicans* yeast forms were incubated in GBE medium with or without isoproterenol or dB-cAMP. After 120 min, quadruplicate aliquots were removed and 400 cells were scored for yeast and germinating forms. In the absence of isoproterenol or dB-cAMP, 50% of the yeast cells underwent germination. The values given are from two separate experiments.

transition of this organism. This suggests that alterations in cAMP may not simply be a correlate of this transition but, instead, may be a trigger for morphogenesis.

PGE_2, at levels now shown to promote *Candida* germination, has previously been identified in the vaginal fluids of women with recurrent candidal vaginitis (23). It was postulated that PGE_2-mediated inhibition of cell-mediated immune responses, which leads to a reduction in IFN-γ production (4, 7, 11, 14), decreased the ability of phagocytes and cytotoxic lymphocytes to limit *Candida* proliferation. It can now be proposed that PGE_2 also increases *Candida* infectivity by inducing the yeast-to-hyphal transition. The hyphal form of this organism is better able to adhere to mucosal cells than is the yeast form (20) and, thus, is more likely to invade host tissues and initiate clinical disease.

In women with intact cellular immunity, *Candida*-induced IFN-γ production leads to immune system activation and elimination of the organism. The demonstration that IFN-γ also directly inhibits *Candida* germination provides an additional mechanism whereby the immune response limits pathogenicity. The observation that products of activated lymphocytes inhibit *Candida* germ tube formation has been made previously (J. D. Sobel and M. Opitz, Abstr. Annu. Meet. Intersci. Conf. Antimicrob. Agents Chemother. Abstr. 581, p. 198, 1987).

The mechanism of IFN-γ inhibition of *Candida* germination remains to be elucidated. One intriguing possibility, currently under investigation, is that *C. albicans* possesses specific receptors for human IFN-γ and that this interaction inhibits cAMP production in this organism. *C. albicans* does possess specific receptors reactive with human progesterone and corticosterone (15) and complement components C3d and iC3b (12). In addition, fibrinogen, albumin, and transferrin also bind to this organism, by undetermined mechanisms (19).

Candida vaginitis most often occurs during the luteal phase of the menstrual cycle (13), when cell-mediated immune responses to *C. albicans* are at their lowest level (A. Kalo-Klein and S. S. Witkin, Am. J. Obstet. Gynecol., in press). Under these conditions, IFN-γ production is decreased. Conversely, PGE_2 levels increase during the luteal phase due to progesterone stimulation of uterine prostaglandin production (1, 8, 16). Similarly, immunosuppressed individuals may exhibit increased levels of PGE_2 or decreased levels of IFN-γ in their circulation. These alterations increase the ability of *C. albicans* to form germ tubes and initiate a clinical infection. Immunosuppressive drugs, by elevating cAMP production (3), can also increase susceptibility to candidiasis by a similar mechanism.

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