INFECTION AND IMMUNITY, Jan. 2002, p. 400–402 Vol. 70, No. 1
0019-9567/02/$04.00+0 DOI: 10.1128/IAI.70.1.400–402.2002

Production of Prostaglandins and Leukotrienes by Pathogenic Fungi

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Received 29 June 2001/Returned for modification 9 August 2001/Accepted 27 September 2001

These studies demonstrate that pathogenic fungi (dermatophytic, subcutaneous, and systemic) have the ability to produce eicosanoids both from simple metabolites and from arachidonic acid. Host-derived eicosanoids have been previously demonstrated to enhance fungal colonization and atopic disease development. Thus, fungus-derived eicosanoids represent a potential class of novel virulence factors.

Eicosanoids are potent regulators of host immune responses (14). Eicosanoids are oxygenated metabolites of dihomo-γ-linolenic, arachidonic, or eicosanopentaenoic acid and include the prostaglandins and leukotrienes. Prostaglandins (e.g., prostaglandin D_2 [PGD_2], PGE_2, and $PGF_{2\alpha}$) are produced via the initial action of prostaglandin G/H synthase (cyclooxygenase) followed by specific prostaglandin synthases. Leukotrienes are produced via a lipoxygenase (LOX) followed by conversion into leukotriene B_4 (LTB_4) and the cysteinyl leukotrienes (LTC_4, LTD_4, and LTE_4) by different synthases and hydrolases. Prostaglandins can inhibit Th1 type immune responses, chemokine production, phagocytosis, and lymphocyte proliferation (3, 9, 11, 14, 17, 19, 20). Leukotrienes are potent leukocyte chemotactic factors (7). Prostaglandins and leukotrienes can also promote Th2 type responses and tissue eosinophilia (4, 7, 11, 14, 18). In the context of antifungal immunity, chronic or disseminating fungal infections will result if the early Th1/Th2 balance of cellular immunity is shifted away from Th1 toward Th2 type responses (16). Thus, enhanced prostaglandin production during fungal infection could be an important factor in promoting fungal colonization and chronic infection.

Host cells are one source of eicosanoids during fungal infection; however, another potential source of eicosanoids is the fungal pathogen itself. We recently reported that the pathogenic yeasts *Cryptococcus neoformans* and *Candida albicans* produce prostaglandins de novo and when fed exogenous arachidonic acid (AA) (13). In addition, yeast-derived PGE could inhibit lymphocyte proliferation, decrease tumor necrosis factor alpha production, and augment interleukin-10 production (13). Our objective was to determine if eicosanoid production extended to other pathogenic fungi, including dermatophytes (*Epidermophyton floccosum*, *Fusarium dimerum*, *Microsporum audouinii*, *Microsporum canis*, and *Trichophyton rubrum*), subcutaneous pathogens (*Sporothrix schenckii*), and systemic pathogens (*Absidia corymbifera*, *Aspergillus fumigatus*, *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Penicillium* spp., *Rhizopus* spp., and *Rhizomucor pusillus*).

To examine eicosanoid production in various species of pathogenic fungi, PGE_2, PGD_2, $PGF_{2\alpha}$, LTB_4, and cysteinyl leukotriene (CysLT) levels from culture supernatants were measured. *H. capsulatum* and *B. dermatitidis* were grown for 7 days at 37°C in RPMI medium while shaking. All others were grown for 7 days at 25°C in RPMI medium while shaking. The cultures were incubated for an additional 2 h with 1 mM AA (Cayman Chemicals, Ann Arbor, Mich.). Culture supernatants from both AA-fed and non-AA-fed fungi were analyzed for eicosanoid production using enzyme-linked immunosorbent assay kits (Cayman Chemicals) for PGE_2, PGD_2, $PGF_{2\alpha}$, LTB_4, and CysLT (detects LTC_4, LTD_4, and LTE_4) according to the manufacturer's instructions. The cultures without AA measure the (endogenous) production of eicosanoids by fungi in the absence of exogenous fatty acid substrates (RPMI medium is a defined medium devoid of fatty acids). All fungal strains grown in RPMI medium alone produced PGE_2, PGD_2, $PGF_{2\alpha}$, LTB_4, and CysLT (Table 1). In the presence of exogenous AA, approximately 10-fold more of each eicosanoid was detected in the cultures (Table 2). *C. neoformans* and *C. albicans* also produced both prostaglandins and leukotrienes from exogenous AA. These data demonstrate that all of these pathogenic fungi have the ability to convert exogenous AA into both LOX- and prostaglandin G/H synthase-derived eicosanoids. Thus, pathogenic fungi have the ability to produce eicosanoids both from simple metabolites and from exogenous AA.

Fungal infections are most notable for their chronicity (e.g., dermatophyte infections) and nonprotective or injurious inflammatory responses (e.g., dermatophytoses, subcutaneous mycoses, and vaginal candidiasis). In mammals, prostaglandins and leukotrienes can play a dual role in the pathogenesis of inflammatory diseases, both promoting and counteracting inflammatory processes (7, 12). All fungal pathogens examined produced prostaglandins and leukotrienes in the absence and presence of extracellular AA (Tables 1 and 2), thereby representing the potential for both de novo and "trans-species" metabolic production of eicosanoids during infection (during infection, exogenous AA could be generated via the action of fungal phospholipases on host phospholipids) (5). Fungal leukotrienes could enhance the acute phase of an inflammatory response, while fungal prostaglandins could locally down-regulate the innate effector phase or protective Th1 response to the infection. The result of fungal eicosanoid production would be an immunologic "tolerance" of the infection with or without

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TABLE 1. Eicosanoid levels in culture supernatants of pathogenic fungi[a]

Fungal species	Strain[b]	Mean (SE) eicosanoid level (pg/ml)				
		Prostaglandins			Leukotrienes	
		PGE_2	PGD_2	$PGF_{2\alpha}$	CysLT	LTB_4
Absidia corymbifera	ATCC 66271	67 (1)	109 (28)	12 (1)	256 (17)	21 (2)
Aspergillus fumigatus	ATCC 13073	75 (19)	248 (140)	4 (1)	230 (89)	12 (3)
Blastomyces dermatitidis	UM	113 (8)	144 (31)	8 (2)	273 (11)	21 (3)
Epidermophyton floccosum	ATCC 52061	76 (22)	115 (25)	3 (1)	204 (2)	15 (1)
Epidermophyton floccosum	UM	66 (5)	79 (20)	2 (1)	207 (13)	8 (0)
Fusarium dimerum	ATCC 62876	24 (5)	35 (14)	6 (1)	29 (9)	3 (1)
Histoplasma capsulatum	UM	86 (0)	100 (4)	13 (0)	241 (78)	25 (4)
Microsporum audouinii	UM	39 (9)	90 (0)	6 (2)	229 (68)	30 (1)
Microsporum canis	ATCC 42559	22 (16)	111 (24)	4 (1)	168 (33)	13 (1)
Penicillium citrinum	ATCC 76113	121 (24)	119 (14)	7 (0)	32 (17)	17 (2)
Penicillium notatum	ATCC 24655	36 (2)	123 (33)	9 (1)	30 (10)	24 (3)
Penicillium piscarium	ATCC 12109	74 (38)	128 (12)	7 (2)	18 (5)	7 (1)
Penicillium spp.	UM	72 (25)	127 (18)	9 (0)	29 (5)	5 (1)
Rhizomucor pusillus	ATCC 36606	40 (14)	78 (34)	19 (1)	44 (1)	19 (2)
Rhizopus spp.	UM	30 (11)	132 (13)	11 (2)	12 (0)	7 (2)
Sporothrix schenckii	ATCC 24646	137 (2)	164 (1)	9 (1)	33 (5)	9 (2)
Sporothrix schenckii	UM	111 (0)	100 (1)	10 (0)	37 (9)	14 (1)
Trichophyton rubrum	ATCC 18760	81 (22)	146 (1)	5 (1)	7 (6)	12 (3)
Trichophyton rubrum	UM	95 (6)	220 (43)	2 (0)	6 (0)	14 (2)

[a] Fungi were cultured for 7 days in RPMI medium (which does not contain any fatty acids or eicosanoids).
[b] American Type Culture Collection catalog number or reference standard from The University of Michigan Medical Microbiology Laboratory (UM).

acute inflammation in an otherwise immunocompetent host, leading to chronic fungal colonization.

Why do fungi produce eicosanoids? Since the fungi reported in this work represent a diverse group of organisms, one possibility is that there is a link between eicosanoid production and fungal growth. We previously reported that indomethacin and etodolac (prostaglandin G/H synthase inhibitors) could inhibit the growth of *C. neoformans* and *C. albicans* (13). We have also observed that nordihydroguaiaretic acid (a LOX inhibitor) can also inhibit the growth of these fungi (data not shown).

The precise role of fungal eicosanoids in the pathogenesis of fungal diseases remains to be determined, but the potential link between fungal eicosanoids and the development of atopic

TABLE 2. Eicosanoid levels in culture supernatants from arachidonic acid-fed pathogenic fungi[a]

Fungal species	Strain[b]	Mean (SE) eicosanoid level (pg/ml)				
		Prostaglandin			Leukotriene	
		PGE_2	PGD_2	$PGF_{2\alpha}$	CysLT	LTB_4
Absidia corymbifera	ATCC 66271	2,044 (48)	2,068 (425)	996 (129)	4,832 (683)	1,679 (266)
Aspergillus fumigatus	ATCC 13073	2,413 (58)	1,192 (202)	1,611 (300)	1,398 (290)	379 (19)
Blastomyces dermatitidis	UM	1,720 (141)	1,121 (243)	839 (142)	987 (142)	467 (9)
Epidermophyton floccosum	ATCC 52061	1,302 (170)	1,733 (360)	1,736 (176)	3,233 (442)	942 (0)
Epidermophyton floccosum	UM	617 (98)	1,002 (224)	782 (85)	1,427 (79)	356 (38)
Fusarium dimerum	ATCC 62876	1,262 (45)	514 (83)	534 (129)	382 (77)	142 (23)
Histoplasma capsulatum	UM	1,825 (171)	1,211 (306)	973 (239)	1,951 (213)	869 (89)
Microsporum audouinii	UM	1,886 (111)	1,511 (327)	1,516 (205)	2,130 (376)	777 (53)
Microsporum canis	ATCC 42559	345 (67)	1,120 (159)	762 (159)	1,198 (240)	330 (47)
Penicillium citrinum	ATCC 76113	1,073 (144)	662 (4)	954 (257)	346 (48)	387 (31)
Penicillium notatum	ATCC 24655	454 (98)	1,179 (128)	891 (157)	491 (72)	564 (111)
Penicillium piscarium	ATCC 12109	1,148 (70)	1,434 (19)	1,480 (432)	1,337 (169)	910 (81)
Penicillium spp.	UM	1,413 (281)	822 (31)	1,096 (116)	566 (159)	496 (21)
Rhizomucor pusillus	ATCC 36606	378 (35)	261 (79)	3,250 (250)	467 (83)	186 (54)
Rhizopus spp.	UM	868 (133)	1,139 (88)	750 (298)	592 (127)	406 (86)
Sporothrix schenckii	ATCC 24646	1,755 (21)	1,833 (87)	1,015 (34)	1,677 (278)	1,280 (50)
Sporothrix schenckii	UM	690 (171)	961 (28)	776 (42)	629 (131)	498 (40)
Trichophyton rubrum	ATCC 18760	970 (109)	1,409 (76)	1,189 (371)	534 (24)	612 (81)
Trichophyton rubrum	UM	2,287 (244)	1,716 (567)	1,180 (132)	2,304 (583)	1,082 (171)
Cryptococcus neoformans[c]	52D	211 (42)	814 (165)	373 (92)	508 (141)	139 (26)
Candida albicans[c]	CHN1	23 (10)	562 (41)	325 (45)	726 (132)	28 (7)

[a] Fungi were cultured for 7 days in RPMI medium, and then 1 mM AA was added for 2 h.
[b] American Type Culture Collection catalog number or reference standard from The University of Michigan Medical Microbiology Laboratory (UM).
[c] *C. neoformans* and *C. albicans* were cultured for 3 days in Sabouraud dextrose broth, spun out, resuspended in RPMI medium containing 1 mM AA, and incubated for 2 h.

diseases in the host is intriguing. The role of eicosanoids in the pathogenesis of allergy and asthma is well documented (2–4, 7, 8, 11, 13, 14, 16, 21). Fungi elicit a variety of allergic diseases, including asthma, sinusitis, allergic bronchopulmonary mycoses, and hypersensitivity pneumonitis (6, 10, 15). As demonstrated in this study, fungi produce immunologically active mediators that can promote the development and manifestation of atopic responses to the infection itself or fungal antigens. By analogy, schistosome-derived PGD_2 was recently reported to play a role in the immune deviation that occurs following skin infection by this parasite (1). Thus, the discovery that pathogenic fungi produce eicosanoids opens up a new realm of investigation for virulence mechanisms in fungal pathogenesis and also for fungal eicosanoids as potential cofactors of atopic diseases.

We thank Susan Salo and Carl Pierson of the University of Michigan Medical Microbiology Laboratory for providing the fungal reference standards and Deirdra Williams and Cara Chrisman for technical assistance.

This work was supported by a New Investigator Award in Molecular Pathogenic Mycology from the Burroughs-Wellcome Fund (G.B.H.). M.C.N. is also supported by NIH-NIAID training grant T32AI07528.

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Editor: T. R. Kozel