

296 Inhibition of Neutrophil Oxidative Burst by *Scedosporium prolificans* and *Aspergillus fumigatus* hyphae

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Scedosporium prolificans (SP) is an emerging opportunistic mould that causes serious infections in immunocompromised patients. Its effect on the oxidative burst of human neutrophils (PMN) was evaluated and compared to that of *Aspergillus fumigatus* (AF). PMN were purified from blood of healthy adults by dextran sedimentation/ficoll centrifugation. For production of membrane fragments, SP and AF hyphae were grown in Yeast Nitrogen Base medium, then incubated in Ethanol/PBS at 4°C for 24 hr and washed. For opsonization, hyphae were incubated with 50% human serum at 37°C for 30 min and washed. Supernatants (Sup) were prepared by incubating 10⁸ conidia/ml in RPMI-1640 for 7 days and then filtering. Superoxide anion (O₂⁻) production was assessed using a Cytochrome C reduction assay. N-Formyl-Methionyl-Leucyl-Phenylalanine (FMLP) was used for PMN stimulation. nM O₂⁻ produced by 10⁶ PMN/ml during 1 hr is shown in the table. α, β, γ ; p<0.05. While unopsonized AF hyphae inhibit oxidative burst by means of Sup only, SP inhibits PMN oxidative burst both by membrane constituents and Sup. The differential inhibitory effects of SP and AF may contribute to the pathogenesis of their infections and may explain differences existing between them.

PMN Only	Treated PMN	SP	AF
0.84 α	Unopsonized fragments	0.57 α	0.95
	Opsonized fragments	2.51	2.16
PMN Only	PMN + Sup	SP	AF
	1.11 β	Sup 0.01%	0.74
	Sup 10%	0.97	0.70 β
PMN + FMLP	PMN + FMLP + Sup	SP	AF
	2.47 γ	Sup 0.01%	2.78
	Sup 10%	1.35 γ	1.00 γ

299 Penicillium marneffeii recombinant antigen Mplp and penicilliosis marneffeii in HIV and non-HIV patients

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Background: *Penicillium marneffeii* is an endemic mycosis in Southeast Asia considered to be an AIDS-defining infection. The Mplp is a *P. marneffeii*-specific mannoprotein. Objectives: The clinical features of the infection in HIV and non-HIV patients are compared and the role of Mplp-based serological test in these two groups of patients was studied. Methods: Patients with culture-documented penicilliosis marneffeii from 1994 to 1999 were included. Detection of *P. marneffeii* antigen (Mplp) and antibody in serum was performed using an ELISA test with plates coated with guinea pig anti-Mplp antibody and Mplp respectively. Clinical and laboratory characteristics of the patients were compared between the HIV and non-HIV patients. Results: 15 cases were available for analysis. HIV positive cases (8) were more likely to have fungemia than non-HIV cases (7). The latter often required tissue biopsy for diagnosis. There was a significant delay in making a diagnosis in non-HIV cases: 1.6 wk vs 6.5 wk (mean). Penicilliosis marneffeii-related mortality was ~. Serum Mplp antigen and antibody titers increased 30 days (mean) before the day of positive culture. In the absence of relapse, titers remained negative for over 1000 days. HIV cases had a higher antigen titer and a lower antibody titer, while the converse is true in non-HIV cases. Conclusions: Except in one, all non-HIV cases have underlying diseases including hemic malignancies and immunosuppressive therapy. 28.6% of non-HIV cases didn't have fungemia at presentation. The major problem associated with the use of polyclonal antibodies or antigens in diagnosing systemic mycoses is specificity. Monoclonal antigens and antibodies are preferable. The *MPL* gene is highly specific for *P. marneffeii*. Mplp-based antigen and antibody assay is a useful adjunct to the diagnosis of penicilliosis marneffeii.

297 Toll-Like Receptors mediate intracellular signaling in response to *Cryptococcus neoformans* polysaccharide capsule.

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C. neoformans causes life-threatening infections among individuals with defects in cellular immunity. Cryptococcal polysaccharide capsule is a major virulence factor and is composed largely of glucuronoxylomannan (GXM). Toll-Like Receptors (TLR) 2 and 4 are cell surface receptors that, often in association with CD14, enable phagocytic inflammatory responses to a variety of microbial products. Activation via these receptors leads to nuclear translocation of NF- κ B and TNF α production. The aim of this study was to investigate whether TLRs participate in the immune response to *C. neoformans*. Chinese hamster ovary (CHO) fibroblasts transfected with TLR2, TLR4 and/or CD14 were incubated with fluorescent bound GXM and analyzed by flow cytometry. Cellular binding of GXM was enhanced in the presence of each of these cell surface receptors. CHO cells were challenged with GXM and the activation of an NF- κ B dependent reporter construct was evaluated. NF- κ B activation was observed in a dose dependent fashion in response to GXM (62.5 – 250 μ g/ml) among cells transfected with both CD14 and TLR4. GXM stimulated nuclear NF- κ B translocation in human peripheral blood mononuclear cells (PBMC) and the murine macrophage cell line, RAW264.7. Challenge of PBMC and RAW264.7 cells with 250 μ g of GXM/ml failed to stimulate TNF α secretion. GXM failed to activate the 3 MAP kinase pathways in PBMC and RAW264.7 cells assessed by Western blotting with antibodies reactive with phosphorylated ERK1/2, p38 and SAPK/JNK. These findings suggest that TLRs, perhaps in conjunction with CD14, function as pattern recognition receptors for cryptococcal GXM. Furthermore, TLR4 appears to mediate intracellular signaling in response to GXM. Finally, the observation that GXM induces NF- κ B translocation, but does not activate MAP kinase pathways, may explain the absence of a TNF α response, and have implications regarding the role of GXM as a virulence factor.

298 Successful treatment of a patient with prosthetic joint arthritis due to a novel yeast characterized by 26S ribosomal DNA sequencing

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Optimal management of fungal infections after total joint arthroplasty is unknown. We report a 51-year-old man with prosthetic knee infection due to a novel yeast successfully treated with joint removal and oral fluconazole. The patient reported pain and swelling for one year after total knee replacement. Multiple joint aspirates yielded an unidentified yeast. The prosthetic joint was removed and the yeast was isolated from intraoperative cultures. Fluconazole 600 mg by mouth was given daily for six months and subsequent cultures of joint fluid were sterile. Minimal inhibitory and fungicidal concentrations of fluconazole against the organism were both 8.0 μ g/ml. Serum fluconazole concentrations were >19.0 μ g/ml at several timepoints. The patient received a second prosthetic joint three months after finishing therapy. One year later, he remains asymptomatic with a functional knee. The novel yeast initially grew on blood and chocolate agar after twenty-four hours of incubation. Subcultures grew on Emmons' modified Sabouraud glucose agar, fungus selection agar containing cycloheximide, V8 juice agar and yeast malt agar, but not on brain heart infusion agar. Commercial yeast identification systems were unable to identify the organism, but showed that it assimilated glucose, glycerol, xylose, erythritol, adonitol, xylitol, sorbitol, cellobiose, trehalose. The organism was able to assimilate nitrate, but was unable to hydrolyze urea. It produced round to oval blastoconidia but no hyphae, pseudohyphae, or ascospores. Sequencing of the D1/D2 domain of large subunit (26S) ribosomal DNA showed sequence divergence of >1% from related yeasts, indicating a new species. The isolate was most closely related to *Pichia angusta*, differing by seven nucleotides. Final identification and designation of this yeast as a new species in the genus *Candida* or *Pichia* depends on tests now underway to detect its ability to form ascospores after prolonged incubation. Our case illustrates the successful treatment of prosthetic arthritis due to a novel yeast using joint removal and oral fluconazole.

300 Zinc-reversible antimicrobial activity of recombinant migration inhibitory factor related proteins 8 and 14.

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Abscess fluid supernatants and neutrophil lysates have zinc-reversible microbial growth-inhibitory activity mediated by a calcium and zinc-binding protein called the migration inhibitory factor-related proteins 8 and 14 (MRP 8 & 14) or calprotectin. In this study recombinant MRP 8 and MRP 14 chains were tested for antimicrobial activity in a *Candida albicans* growth inhibition assay. Both chains contain HEXXH zinc-binding sites and might be expected to manifest zinc-reversible antimicrobial activity ~ to that of the native protein complex. When tested alone, neither MRP 8 nor MRP 14 showed activity in the *Candida* growth assay; a synthetic 20 amino acid peptide containing the HEXXH sequence of MRP 14 along with a nearby HHH sequence was also inactive in this assay (all three preparations not producing growth inhibition at >50 μ M concentrations in 4 experiments per point). However, equimolar concentrations of MRP 8 and MRP 14 demonstrated potent growth-inhibitory activity in this assay (50% inhibition at 3.2 \pm 0.3 μ M and 90% inhibition at 4.6 \pm 0.1 μ M in 7 experiments per point). This growth inhibition was completely inhibited by addition of zinc at 30 μ M. A truncated form of MRP 14 (missing the C-terminal GHHKPLGEGTPT tail) used in combination with MRP 8 demonstrated zinc-reversible activity somewhat less than that with complete MRP 14. These results suggest that a heterodimer of MRP 8 and MRP 14 is necessary to form a zinc-binding site capable of inhibiting microbial growth and that the HHH containing MRP 14 tail is of lesser significance to this activity.

301 Endogenous Retinoid Levels After Voriconazole Administration

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Voriconazole (V) is a novel triazole antifungal agent with potential broad spectrum applications. Adverse reactions in patients receiving V for treatment of fungal infections include rash (10%) (Swift and Denning (1998) J Otol Laryngol 92:97) and cheilitis (rarely). A possible mechanism is that elevations in endogenous retinoid levels are linked to these reactions, since clearance of retinoid acids is mediated by CYP3A4, which V inhibits. To investigate this further, retinoids were measured in 2 studies. In Study 1, retinol (R) and all-trans and 13-cis retinoic acids (RA) were measured after a single i.v. dose of V (8 mg/kg) or placebo was administered to 8 healthy volunteers, in a cross over design. After a single 8 mg/kg dose, V had no effect on R or 13-cis RA. All-trans RA levels were mildly elevated (table), but stayed within normal levels (<5ng/ml) and returned to baseline within 12 hours. In Study 2, R and retinoid binding protein (RBP) were assayed after 14-days of oral administration (V: 200 mg bd (N=9) and 300 mg bd (N=9); fluconazole 400 mg od (N=6)) to patients at risk for aspergillosis. R and RBP levels were unaffected by 14-days of voriconazole or fluconazole treatment. Neither rash or cheilitis were reported in either Study 1 or Study 2. Overall, voriconazole had no effect on R, RBP or 13-cisRA. A transient effect on all-trans RA levels, which returned to baseline levels, was detected after a single dose, however elevation of endogenous retinoids in V-associated rash was not substantiated.

All-trans retinoic acid concentrations (ng/ml) after single voriconazole dose

Time (hrs)	Placebo (N=8)	Voriconazole (N=8)
0	1.4 \pm 0.1	1.1 \pm 0.1
1	1.6 \pm 0.1	2.0 \pm 0.3
4	1.7 \pm 0.1	2.6 \pm 0.3
8	1.4 \pm 0.1 (N=7)	1.5 \pm 0.1
12	1.3 \pm 0.1 (n=7)	1.1 \pm 0.1