

XANTHINE METABOLISM IN CRYPTOCOCCUS NEOFORMANS VAR. GATTII VIRULENCE

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In immuno-competent human and mouse populations, CNS and other organ manifestations of infection by *C. neoformans* var. *gattii* appear more clinically severe and difficult to treat than those with var. *neoformans* (1,2). Infections with var. *gattii* are infrequent among immuno-compromised human populations highly susceptible to var. *neoformans* infections (3). Bases for the differences in virulence are not well understood, but phenol oxidase activity is linked to var. *neoformans* virulence. The oxidoreductase enzymes of xanthine metabolism have broad substrate specificity for heterocyclic enols and aldehydes. The expression of the xanthine catabolic pathway in clinical isolates of *C. neoformans* var. *neoformans* has been characterized and compared to expression in zygomycetous fungal agents of various Rhino-cerebral infections(4,5,6). All of these grow on defined media with hypoxanthine as sole nitrogen source, and the xanthine oxidase(s), with FAD and Molybdopterin cofactors, are concentrated in large microbodies, readily detected and characterized by microscopic analyses. Molecular probes correlated virulence among strains of *Rhizopus* with strong expression of the scavenging pathway culminating in xanthine oxidase expression(6). WE ASK:

Are isolates of *C. neoformans* var. *gattii* from clinically severe infections upregulated for expression of the xanthine catabolic pathway; are they more able to sustain growth on defined media with hypoxanthine or xanthine as sole nitrogen source, than environmental isolates of *C. neoformans* var. *gattii*? Previously described methods were used for TEM, SEM-EDX-ray (5) and Confocal-autofluorescence-microscopic (A.M. Fiskin and M.R. McGinnis, pers. comm.) analyses of microbodies dedicated to xanthine catabolism. For K.C. clinical isolates of *C. neoformans* var. *neoformans*, growth on xanthine as sole nitrogen source or sucrose as sole carbon source was demonstrated, with the production of fluorescent microbodies expressing xanthine(molybdopterin)- or hexose-oxidase(s) activity, resp. For these preliminary studies the Mycology Unit, Adelaide, provided in blind 4 environmental and 4 clinical isolates, including some number of var. *neoformans*, as distilled water suspensions. Each isolate was diluted to a turbidometric standard in defined media, including trace elements, Mo, Fe, and Cu, with either malic acid and urea (permissive), sucrose and urea, malic acid and xanthine, or malic acid and xanthine plus micro-molar urea; as sole sources of carbon and nitrogen, resp. Time to grow 2X and 4X was recorded, then 1G pellets were transferred to para-formaldehyde fixative for 4 hr., then examined by Confocal-microscopy or transferred to gluteraldehyde overnight, then post-fixed briefly with osmium tetroxide, for electron-microscopic analyses.

Among the environmental isolates, only the 1 var. *neoformans* isolate grew on xanthine as sole nitrogen source. All 4 grew on sucrose as sole carbon source, with fluorescent microbodies without molybdopterin, and all grew on permissive medium, with no fluorescent microboies. The 3 isolates that did not grow on xanthine alone all grew slowly on xanthine plus urea, with microbody production at a lower level. All 4 clinical isolates grew profusely on xanthine as sole nitrogen or sucrose as sole carbon sources, with expression of the resp. microbody enzymes. The 1 clinical isolate identified as var. *neoformans* grew most rapidly on sucrose as sole carbon source, but grew on xanthine as sole nitrogen source no more rapidly than var. *gattii* Too few isolates were analyzed for definitive conclusions, but the results are consistent with upregulation of molybdopterin and xanthine catabolic enzymes during progression of var. *gattii* infections. Such studies can readily be repeated now on large numbers of isolates. If our results are confirmed, we hope to test in the mouse model virulence of isolates that cannot be accommodated to grow on xanthine as sole nitrogen source, and to characterize proteins regulating xanthine catabo;ism, using quantitative molecular probes.

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