J. Med. Microbiol. — Vol. 51 (2002), 189–191

ISSN 0022-2615

EDITORIAL

Melanins in fungal pathogens

Melanins are multifunctional polymers that are found throughout nature. They are typically dark brown or black pigments of high mol. wt, although determining their precise structure has proved very difficult. Typically they are formed through the oxidative polymerisation of phenolic compounds. Three principal types of melanin are recognised: eumelanins (formed by a complex polymerisation process involving quinones and free radicals), phaeomelanins (derived from tyrosine and cysteine) and allomelanins (formed from nitrogen free precursors). In plant fungal pathogens melanins have attracted considerable interest as putative virulence factors, and in the last 10 years increasing attention has been directed to the study of melanisation in their human equivalents.

Most of this interest has been directed towards the capsulate yeast *Cryptococcus neoformans* (an important opportunist infection in AIDS patients amongst others) and indeed melanisation was identified in this organism as early as 1962; by the mid-1980s classical genetic studies had implicated melanin production with virulence [1]. The enzyme responsible for the process, a laccase, has been characterised, and the expression of the gene that encodes it (CNLAC1) has been shown to be associated with virulence [2], *via* gene deletion studies. The nature of the melanin produced has been defined and some of the mechanisms by which melanin plays a role in the virulence of *C. neoformans* have been identified (see below). In contrast to some of the fungi described below, *C. neoformans* appears to be incapable of *de novo* melanogenesis and instead relies on the presence of dihydroxyphenolic compounds.

Perhaps most significantly, various reagents have been produced against *C. neoformans* melanin which have allowed the detection of melanisation *in vivo* [3, 4]. Of particular importance has been the production of anti-melanin monoclonal antibodies (MAbs) that are reactive against a wide spectrum of melanin types. With these reagents, it has recently proved possible to detect melanisation for the first time in the Latin American tropical dimorphic pathogen *Paracoccidioides brasiliensis* [5]. Both the conidia (the infectious propagule) and the yeast (*in vivo*) form of this important fungal pathogen appear to produce a type of melanin. Biophysical analysis, by electron spin resonance spectroscopy, was used to confirm the identity of the black pigment produced as melanin and, most convincingly, melanised particles were recovered from infected animal tissue. We also now have additional data that indicates that a second important dimorphic fungal pathogen, *Penicillium marneffei* (which infects AIDS patients in south-east Asia), also melanises in the conidial form (unpublished data). The evidence for this consists of immunological recognition by MAbs, biophysical profiling and detection of an anti-melanin polyclonal response in mice infected with *Pen. marneffei*. The identification of melanin in these two pathogens paves the way for further studies such as those that confirmed the relationship between melanin and virulence in *C. neoformans*.

Whilst the work on *C. neoformans* melanisation is the most comprehensive of that performed on human fungal pathogens, advances have also been made in the study of melanisation in *Sporothrix schenckii*, *Exophiala* (*Wangiella*) *dermatidis* and members of the genus *Aspergillus* (particularly *A. fumigatus*). *S. schenckii*, which produces pigmented conidia and is associated with a predominantly cutaneous/lymphatic disease, has long been suspected of producing melanin, and disruption of the melanisation process results in mutants with attenuated virulence [6]. Similar data are now available for the dermatiaceous fungus *E. dermatitidis*, and the pentaketide pathway used to produce melanin in this fungus has been successfully blocked by the inhibitor tricyclazole [7]. In the case of *A. fumigatus*, a series of genes has been identified, such as polyketide synthase and scytalone dehydratase [8, 9], the disruption of which impairs dihydroxynaphthalene-melanin biosynthesis and lowers conidial virulence. The situation in this organism appears rather more complicated because it appears that there are also secondary effects on conidial architecture when melanisation is disrupted, which may have additional impacts on virulence.

Based on what is known both from studies on the fungal pathogens described above and from work on synthetic melanins, it is possible to develop a model for how fungal melanins may play a role in pathogenesis. Thus melanin may act as:

1) A potent free radical scavenger, protecting the cell against oxidants generated *via* immune effector cells. Thus, non-melanised *C. neoformans* cells may be more susceptible to both oxygen- and

nitrogen-derived oxidants [10]; similar data have been obtained in the case of *S. schenckii*, *E. dermatitidis* and *A. fumigatus*.

2) Non-specific 'body armour', protecting the cell from such diverse insults as UV light, extremes of temperature, antimicrobial peptides and antifungal drugs; such protective effects have been demonstrated in *C. neoformans*. Therefore, by inference, melanised cells are more likely to survive in the external environment, notwithstanding any potential effect *in vivo.* Indeed it is plausible that melanisation in the fungi described here evolved initially to protect cells from external environmental influences rather than in response to the actions of the mammalian immune system.
3) An immunomodulatory substance – synthetic melanin – has been implicated in the suppression of the production of pro-inflammatory cytokines [11], and it is conceivable, although as yet unproven, that melanin may play a similar localised immunosuppressive role during fungal infection.

There also remains the intriguing possibility that melanisation of fungal cells *in vivo* may be related in some way to the phenomenon of reactivation. Proving the occurrence of the latter is difficult, but in *P. brasiliensis* infections, for example, it has always been assumed to be a significant mechanism. Interestingly, from in-vivo studies carried out in this laboratory it would appear that only a proportion of *P. brasiliensis* cells melanise; is it possible that such cells are those which then persist for extended periods and are those which are subsequently responsible for reactivation events? There is also an on-going debate as to the relevance of reactivation in *C. neoformans* infections. This area in particular is worthy of further investigation.

Perhaps the primary motivation underpinning research into virulence determinants is the possibility that such studies may identify novel targets for therapeutic intervention. In view of this, the recent study examining chemical inhibition of in-vivo melanisation in *C. neoformans* by Casadevall's group in New York is of some significance [12]. The authors were able to use the herbicide glyphosate (which is an inhibitor of the shikimate acid pathway) to delay melanisation of yeast cells *in vivo*, and in so doing prolong the survival of mice infected with *C. neoformans*. The herbicide inhibited the autopolymerisation of L-dopa and oxidation of L-epinephrine by cryptococcal cells. Whilst clearly no-one would advocate the use of glyphosate as a treatment for human cryptococcosis, this observation does suggest that there may be some mileage in examining melanin-inhibiting compounds as treatments. However, clearly, as mammals themselves produce melanin (e.g., in the skin), there are obvious concerns regarding precise targeting of such intervention. Another potentially interesting recent observation is that anti-melanin MAbs can be used to prolong the survival of mice infected with *C. neoformans* [13]; previous attempts at passive immunisation have relied on capsular carbohydrate components as targets.

Whilst it should be clear from the previous discussions that melanin may act as a defensive wall surrounding the fungal cell, this must be to some extent a double-edged sword, as this wall must have to be pierced or at the very least reconstructed during phases of growth. Thus, during budding in *C. neoformans*, melanin deposited in the cell wall must be removed or modified in some way. Given that melanin is such a robust substance (which can for instance survive treatment with lytic enzymes, chaotrophic agents and boiling in concentrated HCl), an analysis of the processes (presumably enzymic) leading to melanin removal would be of great interest. Indeed, if melanin degradation is central to cell division in fungi such as *C. neoformans* and *P. brasiliensis*, then it is possible that the processes involved would also make potential therapeutic targets. Indeed, these may perhaps make better targets than the melanin synthesis enzymes mentioned above, given that melanin degradation in mammals is probably not a crucial physiological process.

In summary, recent developments in our ability to detect melanin production, arising largely from work on *C. neoformans*, have been of considerable help in demonstrating that melanisation is a more general phenomenon in fungal pathogens of man than previously thought. Whether melanisation in fungi such as *P. brasiliensis* and *Pen. marneffei* has similar roles to that found in *C. neoformans* (and other fungi such as *E. dermatitidis*) remains to be seen, although the balance of probability is that it does. As such, melanisation may come to be regarded as one of the more widely distributed and important fungal virulence determinants.

A. J. HAMILTON, B. L. GOMEZ
Dermatology Laboratory,
St Johns Institute of Dermatology,
5th Floor, Thomas Guy House,
Guys Hospital,
Kings College,
London SE1 9RT, UK.
Corresponding author: Dr A. J. Hamilton
(e-mail: andrew.j.hamilton@kcl.ac.uk).

References

1. Kwon-Chung KJ, Polacheck I, Popkin TJ. Melanin-lacking mutants of *Cryptococcus neoformans* and their virulence for mice. *J Bacteriol* 1982; **150**: 1414–1421.
2. Salas SD, Bennett JE, Kwon-Chung KJ, Perfect JR, Williamson PR. Effect of the laccase gene CNLAC1, on virulence of *Cryptococcus neoformans*. *J Exp Med* 1996; **184**: 377–386.
3. Rosas AL, Nosanchuk JD, Gomez BL, Edens WA, Henson JM, Casadevall A. Isolation and serological analyses of fungal melanins. *J Immunol Methods* 2000; **244**: 69–80.
4. Nosanchuk JD, Rosas AL, Lee SC, Casadevall A. Melanisation of *Cryptococcus neoformans* in infected human brain tissue. *Lancet* 2000; **355**: 2049–2050.

5. Gomez BL, Nosanchuk JD, Diez S *et al*. Detection of melanin-like pigments in the dimorphic fungal pathogen *Paracoccidioides brasiliensis in vitro* and during infection. *Infect Immun* 2001; **69**: 5760–5767.
6. Romero-Martinez R, Wheeler M, Guerrero-Plata A, Rico G, Torres-Guerrero H. Biosynthesis and functions of melanin in *Sporothrix schenckii*. *Infect Immun* 2000; **68**: 3696–3703.
7. Wheeler MH, Stipanovic RD. Melanin biosynthesis and the metabolism of flaviolin and 2-hydroxyjuglone in *Wangiella dermatitidis*. *Arch Microbiol* 1985; **142**: 234–241.
8. Tsai H-F, Washburn RG, Chang YC, Kwon-Chung KJ. *Aspergillus fumigatus* arp1 modulates conidial pigmentation and complement deposition. *Mol Microbiol* 1997; **26**: 175–183.
9. Tsai H-F, Chang YC, Washburn RG, Wheeler MH, Kwon-Chung KJ. The developmentally regulated alb1 gene of *Aspergillus fumigatus*: its role in modulation of conidial morphology and virulence. *J Bacteriol* 1998; **180**: 3031–3038.
10. Wang Y, Casadevall A. Susceptibility of melanized and nonmelanized *Cryptococcus neoformans* to nitrogen- and oxygen-derived oxidants. *Infect Immun* 1994: **62**: 3004–3007.
11. Mohagheghpour N, Waleh M, Garger SJ, Dousman L, Grill LK, Tuse D. Synthetic melanin suppresses production of proinflammatory cytokines. *Cell Immunol* 2000; **199**: 25–36.
12. Nosanchuk JD, Ovalle R, Casadevall A. Glyphosate inhibits melanization of *Cryptococcus neoformans* and prolongs survival of mice after systemic infection. *J Infect Dis* 2001; **183**:1093–1099.
13. Rosas AL, Nosanchuk JD, Casadevall A. Passive immunization with melanin-binding monoclonal antibodies prolongs survival of mice with lethal *Cryptococcus neoformans* infection. *Infect Immun* 2001; **69**: 3410–3412.