INFECTION AND IMMUNITY, Aug. 1994, p. 3543–3546
0019-9567/94/$04.00+0

Vol. 62, No. 8

NOTES

Altered Expression of Surface α-1,3-Glucan in Genetically Related Strains of *Blastomyces dermatitidis* That Differ in Virulence

LAURA H. HOGAN* AND BRUCE S. KLEIN

Departments of Pediatrics, Internal Medicine, and Medical Microbiology and Immunology, University of Wisconsin Medical School, University of Wisconsin Hospital and Clinics, Madison, Wisconsin 53792

Received 7 March 1994/Returned for modification 7 April 1994/Accepted 28 April 1994

Recent studies of the dimorphic fungal pathogens *Histoplasma capsulatum* and *Paracoccidioides brasiliensis* have suggested a role in virulence for the cell surface carbohydrate α-(1,3)-glucan. To investigate a possible basis for α-(1,3)-glucan in the pathogenicity and virulence of the dimorphic fungus *Blastomyces dermatitidis*, we examined three genetically related strains of *B. dermatitidis* that differ in their virulence for mice: wild-type virulent strain ATCC 26199; mutant strain ATCC 60915, which is 10,000-fold reduced in virulence; and mutant strain ATCC 60916, which is avirulent. Immunologic quantitation of cell wall α-(1,3)-glucan revealed that the mutant yeasts were almost devoid of this sugar moiety, in contrast to the high concentration of α-(1,3)-glucan on the cell wall of the wild-type yeasts. These differences are discussed in relation to previous studies of yeast surface expression of the WI-1 antigen and recognition and binding of the related strains by human monocyte-derived macrophages.

Blastomyces dermatitidis is a thermal dimorphic fungus that can be isolated from soil (3, 4, 9). When the soil is disturbed, aerosolized conidia and hyphal fragments are inhaled by mammals, primarily humans and dogs, and lodge in the lungs, converting to the pathogenic yeast form at 37°C. The yeast typically causes a chronically progressive pneumonia but is also capable of disseminating to the skin, bones, genitourinary tract, and brain. *B. dermatitidis* is mainly a primary pathogen endowed with the ability to invade and cause disease in otherwise normal hosts. Conversion to the yeast form confers a highly selective advantage on survival of the organism (6), but the factors that account for its pathogenicity and virulence remain unknown.

DiSalvo and Denton studied four genetically unrelated strains with differing virulence in mice and found a proportionately higher lipid (but not phospholipid) content in yeast cells of the more virulent strains (5). Cox and Best (2) studied two genetically unrelated strains with differing virulence in mice and found 20-fold more phospholipid associated with the α-linked glucan of the more virulent strain. The disparity between the results of these studies may, in part, reflect the unrelatedness of the isolates studied and the differences in the cell fraction studied.

In the present study, we investigated three genetically related strains of *B. dermatitidis*. The wild-type isolate, strain ATCC 26199, was initially isolated from a patient and is highly virulent in mice (8). Two independent mutants arose spontaneously by serial in vitro passage of wild-type cells: ATCC 60915 is an attenuated mutant, reduced 10,000-fold in virulence for mice (1), and ATCC strain 60916 is completely avirulent (12). When the yeasts are inoculated intranasally into mice, the number of wild-type cells in the lungs increases 10-fold by 48 h postinfection, whereas the mutants are completely eliminated (1). Scanning electron microscopy shows that wild-type and mutant isolates differ markedly in appearance: the surface of wild-type yeasts is rough, whereas the surfaces of the mutants are smooth (1).

The difference in surface roughness between the virulent and avirulent strains of *B. dermatitidis* is reminiscent of recently isolated smooth colony variants of *Histoplasma capsulatum*. Smooth variants of *H. capsulatum* have both reduced surface carbohydrate α-(1,3)-glucan and reduced virulence in mice (11). A decrease in α-(1,3)-glucan content from 45 to 3% is also described for an attenuated mutant of *Paracoccidioides brasiliensis* that arose spontaneously by in vitro passage (13). We therefore investigated surface α-(1,3)-glucan in the genetically related strains of *B. dermatitidis* by using specific monoclonal antibodies (MAbs).

Methods. Strains of *B. dermatitidis* studied were purchased from the American Type Culture Collection and maintained in the yeast form on Middlebrook 7H10 agar slants with oleic acid-albumin complex (Sigma Chemical Co., St. Louis, Mo.) at 37°C.

MAb MOPC 104e (Sigma) was used at a dilution of 1:20 to quantify surface α-(1,3)-glucan expression on the strains (11). Anti-Leu 11b (Becton Dickinson), directed against an irrelevant leukocyte antigen, was used as an immunoglobulin M isotype control for MOPC 104e. For immunofluorescence microscopy, washed unfixed yeast cells (2×10^6/0.1 ml) were incubated for 1.5 h at 37°C with an α-(1,3)-glucan-specific MAb or isotype control antibody in phosphate-buffered saline (PBS) with 1% bovine serum albumin and 0.05% Evans blue counter stain. The yeasts were washed twice with PBS and

* Corresponding author. Mailing address: Clinical Science Center, Room K4/443, 600 Highland Ave., Madison, WI 53792. Phone: (608) 263-6203. Fax: (608) 263-0440.

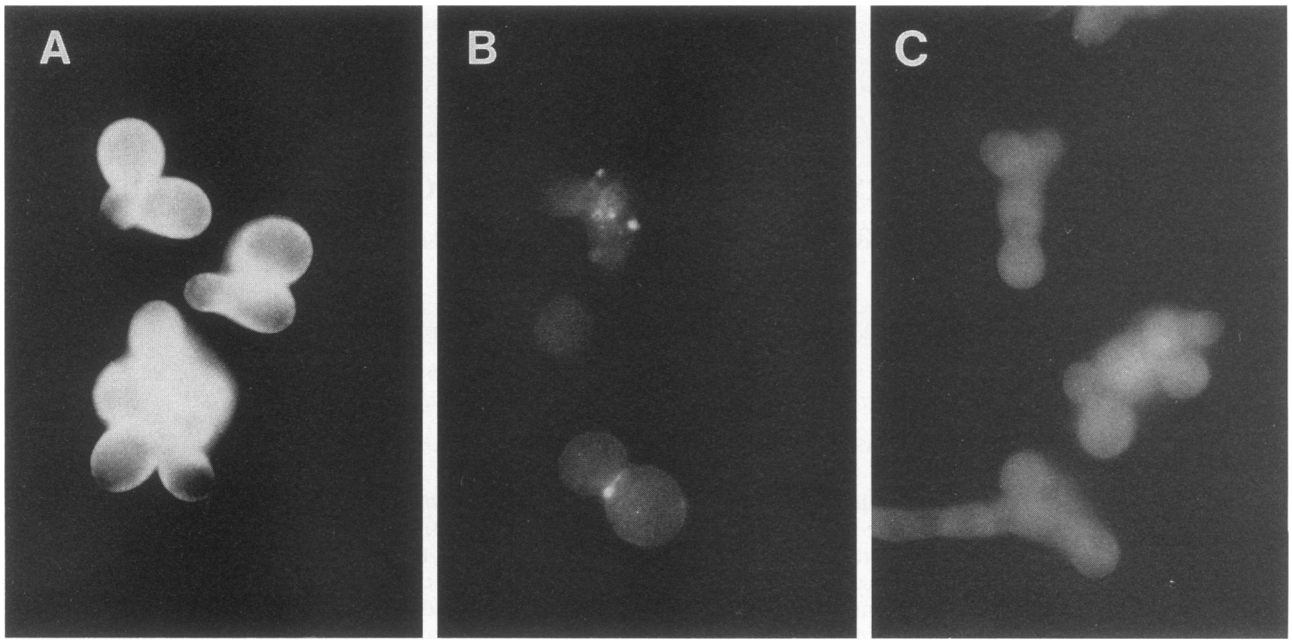

FIG. 1. Cell surface expression of α-(1,3)-glucan measured on three genetically related strains of *B. dermatitidis* yeasts. Binding of α-(1,3)-glucan-specific MAb MOPC 104e was used to assess the amount of yeast cell surface polysaccharide for wild-type strain ATCC 26199 (A) and mutant strains ATCC 60915 (B) and ATCC 60916 (C). Immunofluorescence was assessed microscopically by using an Olympus binocular microscope and a mercury vapor lamp that provided UV illumination. Results are representative of six separate experiments.

incubated with fluorescein isothiocyanate-labeled goat anti-mouse immunoglobulin M (1:20) (Becton Dickinson) for 1.5 h at 37°C. After being washed twice in PBS, the cell pellet was resuspended in 50 μl of PBS. A 5-μl portion of each cell suspension was transferred to a glass slide, covered with a glass coverslip, and examined by fluorescence microscopy with an Olympus binocular microscope with a mercury vapor lamp.

For flow cytometry, fresh yeast cells were grown, harvested, and stained indirectly as described above, except that the Evans blue counterstain was omitted. Stained cells were analyzed by single-color flow cytometry with a four-decade log amplification.

Results. Detection of α-(1,3)-linked glucan on the surface of the yeasts varied markedly among the strains. When probed with MOPC 104e, the wild-type ATCC 26199 exhibited intense, uniform fluorescence over the surface of the cells (Fig. 1). The attenuated mutant strain ATCC 60915 exhibited an irregular staining pattern characterized by highly localized accumulations of intense fluorescence, especially at the site of budding between cells, while the majority of the cell surfaces were not stained. The avirulent mutant strain ATCC 60916 exhibited no fluorescence in this assay. The isotype control antibody did not stain any of the strains tested. As shown, the microscopic morphology of mutant ATCC 60916 differed from that of the other two strains in that a high proportion of cells formed pseudohyphae. Nonetheless, single and budding yeast cells were still devoid of fluorescence. This observation was consistent in six separate experiments.

The relative quantity of α-(1,3)-glucan on the yeast surface of these three strains, when measured by flow cytometry (Fig. 2), was consistent with observations made by microscopy. In two separate flow cytometry experiments, the average values for mean channel fluorescence were as follows: wild-type ATCC 26199, 125; mutant ATCC 60915, 30; and mutant ATCC 60916, 12. Isotype control antibody staining of the yeasts yielded mean channel fluorescence values of 3 to 13, with a mean of 9.

Loss of expression of α-(1,3)-glucan on *P. brasiliensis* yeasts can be partially restored by growth of the fungus in fetal calf serum (14). However, growth of the *B. dermatitidis* mutants for 1 week in brain heart infusion broth containing 15% fetal calf serum did not restore surface α-(1,3)-glucan staining (data not shown). In vivo passage of the *P. brasiliensis* mutant in hamsters restores some production of α-(1,3)-glucan (from 3 to 19.9%) and restores virulence. In previous work by Brass et al., no yeasts could be recovered after infection of mice with *B. dermatitidis* ATCC 60915 or ATCC 60916 (1). In this respect, these *B. dermatitidis* mutants appear to behave similarly to *H. capsulatum* smooth mutants. Neither in vivo passage nor growth in serum-containing medium restores α-(1,3)-glucan production or virulence to *H. capsulatum* mutants (10, 11).

Previous investigators who worked with *H. capsulatum* mutants and *P. brasiliensis* mutants hypothesize that alterations in surface α-(1,3)-glucan may alter the process of phagocytosis, but differences in phagocyte recognition or ingestion of the isolates have not been described. In recent work with these genetically related strains of *B. dermatitidis*, we demonstrated greatly enhanced recognition and binding of the mutant yeasts in vitro by human monocyte-derived macrophages. This enhanced interaction is mediated by the yeast surface antigen WI-1, since expression of the molecule is greatly enhanced on the mutants, and Fab anti-WI-1 MAb blocks the interaction (8a). To these surface alterations in WI-1 expression on the mutants can now be added a reciprocal decrease in surface expression of the carbohydrate α-(1,3)-glucan.

Our experiments do not distinguish between an actual decrease in expression of α-(1,3)-glucan on the mutant cell walls as opposed to a masking of α-(1,3)-glucan from antibody

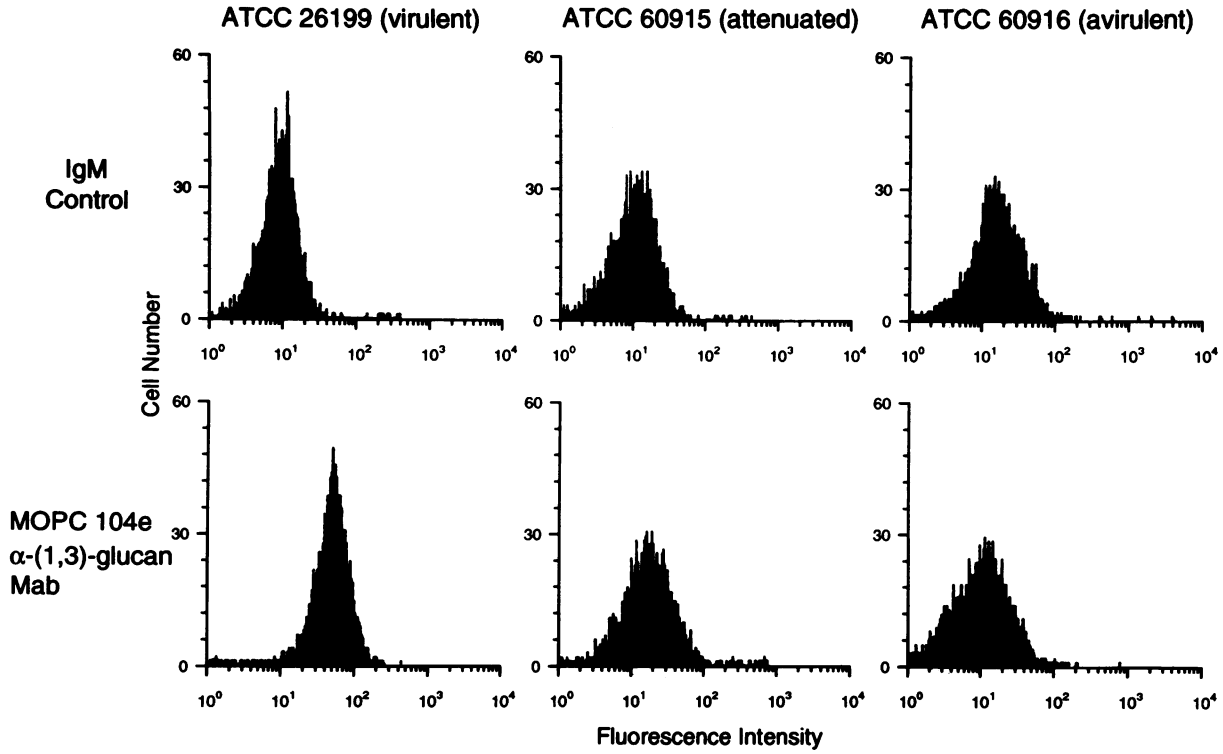

FIG. 2. Cell surface expression of α-(1,3)-glucan measured on three genetically related strains of *B. dermatitidis* yeasts. Binding of α-(1,3)-glucan-specific MAb MOPC 104e was used to quantify the amount of yeast cell surface α-(1,3)-glucan for wild-type and mutant strains. Profiles designated IgM control represent staining with irrelevant isotype control antibody Leu 11b. Results are representative of two separate experiments.

staining by increased expression of WI-1 or other surface molecules. Either mechanism indicates a major alteration in the yeast cell wall architecture accompanying loss of virulence and enhanced binding to human monocyte-derived macrophages. It should be noted that some chemotypes of *H. capsulatum* have greatly decreased α-(1,3)-glucan content but are still virulent (7). Likewise, we have found that not all avirulent strains of *B. dermatitidis* lack staining with the α-(1,3)-glucan-specific MAb (data not shown). *B. dermatitidis* ATCC 26198 (KL-1) does not stain with MOPC 104e. KL-1 was a virulent soil isolate as originally described but has lost virulence over time. The isolate of KL-1 deposited as ATCC 26198 is reported to be avirulent in a mouse model (12). However, another avirulent strain, ATCC 26197 (GA-1), stains well with MOPC 104e. Hence, α-(1,3)-glucan staining does not always correlate with virulence for either *B. dermatitidis* or *H. capsulatum*. Instead, we believe that in wild-type strain ATCC 26199 and in ATCC 26199-derived strains of *B. dermatitidis*, α-(1,3)-glucan may be one component of an array of surface structures which mediates yeast-phagocyte interactions. The glucan polymer may act to modulate WI-1 mediated interactions of the yeast with human macrophages. Experiments are under way to specifically test this role of α-(1,3)-glucan.

We thank John Marx for technical assistance; Henri van der Heyde and the medical media staff at the William S. Middleton Memorial Veterans Hospital for photography and illustration; and Audrey Williamson, Department of Pediatrics, University of Wisconsin Hospital and Clinics, for preparation of the manuscript.

This work was supported by National Institutes of Health grants AI-00905 and AI-31479 to B.S.K. and an NRSA fellowship to L.H.H.

REFERENCES

1. **Brass, C., C. M. Volkmann, D. E. Philpott, H. P. Klein, C. J. Halde, and D. A. Stevens.** 1982. Spontaneous mutant of *Blastomyces dermatitidis* attenuated in virulence for mice. Sabouraudia **20:**145–158.
2. **Cox, R. A., and G. K. Best.** 1972. Cell wall composition of two strains of *Blastomyces dermatitidis* exhibiting differences in virulence for mice. Infect. Immun. **5:**449–453.
3. **Denton, J. F., and A. F. DiSalvo.** 1964. Isolation of *Blastomyces dermatitidis* from natural sites in Augusta, Georgia. Am. J. Trop. Med. Hyg. **13:**716–722.
4. **Denton, J. F., E. S. McDonough, L. Ajello, and R. J. Ausherman.** 1961. Isolation of *Blastomyces dermatitidis* from soil. Science **133:**1126–1127.
5. **DiSalvo, A. F., and J. F. Denton.** 1963. Lipid content of four strains of *Blastomyces dermatitidis* of different mouse virulence. J. Bacteriol. **85:**927–931.
6. **Drutz, D. J., and C. L. Frey.** 1985. Intracellular and extracellular defences of human phagocytes against *Blastomyces dermatitidis* conidia and yeast. J. Lab. Clin. Med. **105:**737–750.
7. **Eissenberg, L. G., and W. E. Goldman.** 1991. *Histoplasma* variation and adaptive strategies for parasitism: new perspectives of histoplasmosis. Clin. Microbiol. Rev. **4:**411–421.
8. **Harvey, R. P., E. S. Schmid, and C. C. Carrington.** 1978. Mouse model of pulmonary blastomycosis: utility, simplicity, and quantitative parameters. Am. Rev. Respir. Dis. **117:**695.

8a. **Klein, B. S., S. Chaturvedi, L. H. Hogan, J. M. Jones, and S. M. Newman.** 1994. Altered expression of surface protein WI-1 in genetically related strains of *Blastomyces dermatitidis* that differ in

virulence regulates recognition of yeasts by human macrophages. Infect. Immun. **62:**3536–3542.

9. **Klein, B. S., J. M. Vergeront, R. J. Weeks, U. N. Kumar, G. Mathai, B. Varkey, and L. Kaufman.** 1986. Isolation of *Blastomyces dermatitidis* in soil associated with a large outbreak of blastomycosis in Wisconsin. N. Engl. J. Med. **314:**529–534.
10. **Klimpel, K. R., and W. E. Goldman.** 1987. Isolation and characterization of spontaneous avirulent variants of *Histoplasma capsulatum*. Infect. Immun. **55:**528–533.
11. **Klimpel, K. R., and W. E. Goldman.** 1988. Cell walls from avirulent variants of *Histoplasma capsulatum* lack α-(1,3)-glucan. Infect. Immun. **56:**2997–3000.
12. **Morrison, C. J., and D. A. Stevens.** 1991. Mechanisms of fungal pathogenicity: correlation of virulence in vivo, susceptibility to killing by polymorphonuclear neutrophils in vitro, and neutrophil superoxide anion induction among *Blastomyces dermatitidis* isolates. Infect. Immun. **59:**2744–2749.
13. **San-Blas, F., G. San-Blas, and L. J. Cova.** 1976. A morphological mutant of *Paracoccidioides brasiliensis* strain IVIC Pb9. Isolation and wall characterization. J. Gen. Microbiol. **93:**209–218.
14. **San-Blas, G., and D. Vernet.** 1977. Induction of the synthesis of cell wall α-1,3-glucan in the yeastlike form of *Paracoccidioides brasiliensis* strain IVIC Pb9 by fetal calf serum. Infect. Immun. **15:**897–902.