INFECTION AND IMMUNITY, Nov. 1985, p. 598–600
0019-9567/85/110598-03$02.00/0

Vol. 50, No. 2

Candida albicans and *Candida stellatoidea*, in Contrast to Other *Candida* Species, Bind iC3b and C3d but Not C3b

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Received 21 March 1985/Accepted 8 August 1985

It was demonstrated that complement-coated sheep erythrocytes bind to *Candida albicans* cells grown in serum-free RPMI 1640 medium. Testing of purified complement components proved that iC3b and C3d were responsible for the reaction, whereas C3b and C3b-H reacted only slightly if at all. Binding occurred only to *C. albicans* and *C. stellatoidea*, not to other species pathogenic to humans. There was evidence of a lectinlike nature of the effect.

On various human cells, such as B lymphocytes, phagocytes, and erythrocytes, binding structures or receptors exist for interaction with sites of the C3 molecule and its fragments C3b, iC3b, and C3d (see reference 6 for a review). We accidentally observed that yeast cells contaminating a lymphoblastoid culture reacted with complement-coated sheep erythrocytes (EAC) in a similar way as lymphoblasts. This was surprising to us, because the cell wall of yeasts has a structure very different from that of the lipoprotein membrane of mammalian cells (2, 7). It consists mainly of a skeleton of β-glucan molecules in which mannan, in linkage to cell wall proteins, plays the role of a cementous substance (10). Thus, so-called mannoproteins are formed, which are typical glycoproteins of well-defined structure (1, 11).

Since yeasts are frequently encountered pathogens, we wanted to define their complement-dependent reaction more precisely and chose *Candida albicans* for investigation, since it is the most common cause of yeast infections in humans. *C. albicans* was compared with less pathogenic *Candida* species.

For the experiments reported below, trypsin from bovine pancreas (40 U/mg), trypsin inhibitor from soybean (35 U/mg), mannan from bakers' yeast, D-glucose, D-galactose, *N*-acetylglucosamine, and D-mannose were bought from Serva, Heidelberg, Federal Republic of Germany; L-mannose was from EGA-Chemie, Steinheim, Federal Republic of Germany; and bovine serum albumin, L-lysine hydrochloride, and glutaraldehyde (20%) were from Sigma Chemical Co., Munich, Federal Republic of Germany. Phosphate-buffered saline (PBS), veronal-buffered saline, veronal-buffered saline containing sucrose, and veronal-buffered saline containing EDTA were composed as previously described (5, 14).

The first component of complement (C1) was prepared from guinea pig serum (4), and all other complement components (C2, C4, and C3), factor H (β1H protein), and factor I (C3b-inactivator protein) were isolated from human serum as previously described (14). The cell culture medium RPMI 1640 was purchased from Flow Laboratories, Inc., Bonn, Federal Republic of Germany, Sabouraud glucose agar was from Merck AG, Darmstadt, Federal Republic of Germany, and glucose broth was from Oxoid Ltd., Wesel, Federal Republic of Germany. Human serum was from normal, healthy individuals. Fetal calf serum was from GIBCO Ltd., Paisley, Scotland.

Sheep erythrocytes were from BAG, Lich, Federal Republic of Germany. They were sensitized (EA) and coated with complement components (EAC) as described elsewhere (14). Glutaraldehyde-fixed erythrocytes were coated with complement by serum incubation (5).

Candida species were isolated from infectious material and identified with the 20C Auxanogramm (API; Mandelieu, Versieu, France). The following species were examined: *C. albicans* (two strains and the two serotypes, A and B), *C. stellatoidea*, *C. tropicalis*, *C. parapsilosis*, and *C. krusei*.

The yeasts were stored in nitrogen at −70°C and grown on Sabouraud agar for 48 h at 37°C. Intermediate storage was at 4°C. The yeasts were harvested from agar cultures, incubated for 48 h at 37°C, and suspended in PBS.

RPMI 1640 (usually in a volume of 30 ml) was inoculated with an initial cell concentration of about 10^5 *Candida* blastospores per ml as calculated from extinction measurements and cell counting with a hemacytometer (for details, see F. Heidenreich, inaugural dissertation, Johannes-Gutenberg University, Mainz, Federal Republic of Germany, 1984). After an incubation time of 20 h in RPMI 1640, the cell concentration was approximately 2×10^6 cells per ml with about 10% pseudomycelial and mycelial forms. Cells were washed twice and suspended in PBS. Cells grown in different media were treated in the same way.

For testing adherence, the following assay was developed. EA or EAC (100 μl; 1.3×10^8 cells per ml of veronal-buffered saline containing sucrose) were mixed with 100 μl of *Candida* suspension (1×10^6 to 3×10^6 cells per ml of PBS). Cells were incubated for 30 min at 37°C in a water bath, washed twice gently, and suspended in PBS. Results were evaluated under a light microscope (magnification, ×40) with a hemacytometer. At least 100 forms were counted, and adherence was defined for two conditions. (i) At least four erythrocytes were bound to a pseudomycelial, mycelial, or hyphal form. Each cell complex was counted as one cell no matter how many areas showed the adherence phenomenon. (ii) Two or more erythrocytes were bound to a single blastospore. Blastospores showing no adherence of erythrocytes at all were neglected owing to difficulties in distinction from erythrocytes. Results were evaluated at least twice. Photographs were taken from characteristic forms with a Zeiss photomicroscope and Ilford Pan F film (80 ASA).

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TABLE 1. Adherence of complement intermediates (EAC coated with purified complement components) to *C. albicans*

Intermediate	Degree of adherence (%)[a]			Qualitative assessment of adherence[c]
	Expt 1[b]	Expt 2[b]	Expt 3[b]	
EA (control)	0	0	0	−
EAC4	0	0	0	−
EAC142°	0	0	0	−
EAC142°3b	1	2	10	(+)
EAC142°3b-H	6	2	10	(+)
EAC142°3bi	31	33	39	+++
EAC142°3d	55[d]	46[d]	51[d]	+++

[a] Expressed as the percentage of *C. albicans* cells showing adherence of EAC.

[b] Each experiment was evaluated three times under the microscope with a hemacytometer, and the mean is reported.

[c] Symbols indicate level of adherence: −, none; (+), very weak; +++, very strong.

[d] Microscopic appearance of the adherence effect was of the same grade for C3d as for iC3b.

We tried to produce inhibition of the adherence effect by preincubating the various cells in different reagents (equal volumes of 100 μl) at 37°C. Further treatment of cells varied. Evaluation was similar to that of the adherence test. However, special notice was given to the microscopic formation of the cell complexes that showed binding of erythrocytes (i.e., number of erythrocytes and number of areas binding erythrocytes).

In preliminary experiments with glutaraldehyde-fixed EAC, it could be demonstrated that complement-dependent adherence to *C. albicans* cells grown in glucose broth, RPMI 1640, and RPMI 1640 containing 5% fetal calf serum occurred qualitatively equally. Quantitative results varied because of different growth characteristics in the different media.

To identify the complement component that was responsible for adherence, we studied complement intermediates (EA coated with purified C4, C2, C3b, C3b-H, and iC3b or C3d or both) with the test systems described above. Results are shown in Table 1 and Fig. 1–4. The low percentage of *C. albicans* cells showing adherence with EAC142°3b or EAC142°3b-H was always due to only a few adhering erythrocytes. For iC3b and C3d, the appearance was clearly different. Microscopic appearance of the effect was of the same strong grade for iC3b as for C3d. Figures 3 (iC3b) and 4 (C3d) are representative of the typical reaction. Figures 3 and 4 are offered at different magnifications to demonstrate an overview and a close-up. Rate and microscopic appearance of the adherence effect corresponded to the C3 load on single erythrocytes (i.e., molecules of iC3b or C3d bound to a single cell). The rate of adherence reached saturation at about 80,000 C3 molecules per erythrocyte.

To rule out restriction of the adherence effect to our initial test strain, we tested another strain and the two serotypes of *C. albicans* and four other *Candida* species for adherence of iC3b and C3d intermediates. All samples of *C. albicans* and *C. stellatoidea* showed qualitatively equivalent adherence. *C. tropicalis*, *C. parapsilosis*, and *C. krusei* did not bind iC3b and C3d intermediates. Apparently, adherence effectively

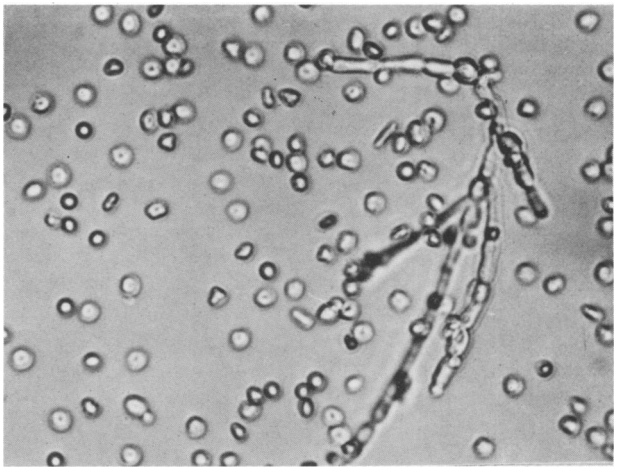

FIG. 2. Incubation of *C. albicans* with EAC142°3b (sheep erythrocytes coated with purified C1, C4, and oxidized C2 and C3b). Only a weak adherence effect is seen (magnification, ×160).

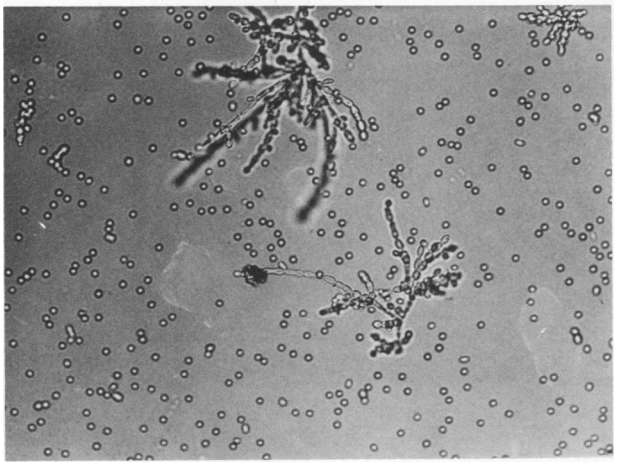

FIG. 1. Incubation of *C. albicans* with EAC142° (sheep erythrocytes coated with purified C1, C4, and oxidized C2). There is no adherence effect detectable (magnification, ×64).

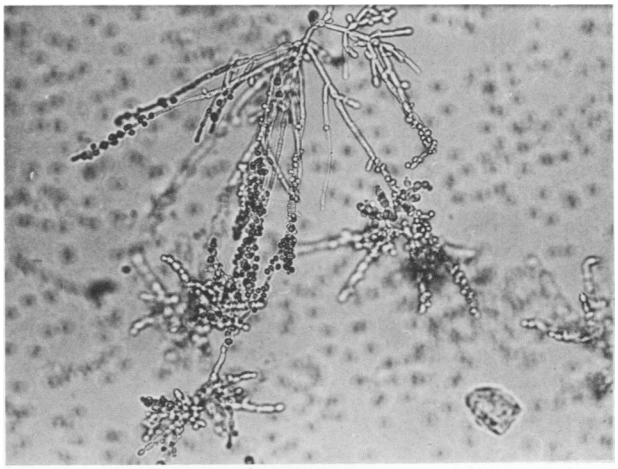

FIG. 3. Incubation of *C. albicans* with EAC142°3bi (sheep erythrocytes coated with purified C1, C4, and oxidized C2 and iC3b). Erythrocytes adhere to the *Candida* cell very strongly and in clusters. Within a single pseudomycelial complex, there are areas that bind erythrocytes and those that do not (magnification, ×40).

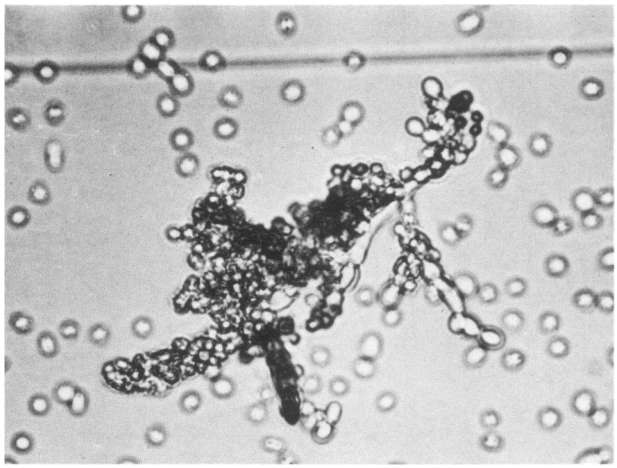

FIG. 4. Incubation of *C. albicans* with EAC142°3d (sheep erythrocytes coated with C1, C4, and oxidized C2 and C3d). Adherence of erythrocytes to the *Candida* cell is very obvious. Clusters of cells cover the *Candida* cell body almost completely (magnification, ×160).

occurred only with intact *C. albicans*, since fragmented material obtained by mechanical grinding and by sonification of yeast cells had only a limited inhibitory effect, as was suggested by the microscopic impression.

Neither mannan from bakers' yeast nor the monosaccharides D-galactose (70 mM), L-mannose (70 mM), and the amino sugar *N*-acetylglucosamine (65 mM) showed a reproducible inhibitory effect on the adherence of iC3b and C3d intermediates. However, preincubation of erythrocytes with D-mannose (70 mM) and D-glucose (70 mM) resulted in inhibition of the adherence of iC3b and C3d intermediates to *C. albicans* of about 30% compared with a control. Inhibition was even more obvious from the poor adherence visible under the microscope.

Thus, we could demonstrate that iC3b- and C3d-coated particles bound to the *C. albicans* cell surface very intensively. Neither a particular growth factor nor fetal serum contained in the medium was necessary for this effect.

From studies performed to characterize the nature of the interaction, there was evidence that the monosaccharides D-glucose and D-mannose could inhibit interaction between iC3b or C3d and *C. albicans*. This suggested a lectinlike nature of the possible interaction between iC3b or C3d and the mannoproteins on the *Candida* cell wall. Comparable results were found for complement-dependent binding to human erythrocytes (13).

Examination of different *C. albicans* strains and serotypes revealed no restriction of reactivity. However, among the different *Candida* species only *C. stellatoidea*, in addition to *C. albicans*, was able to bind iC3b and C3d. *C. stellatoidea* is very closely related to *C. albicans*. Both species form an immunogenically highly active group compared with *C. tropicalis* and other species (9). This is consistent with observations of the pathogenicity of the different species.

It remains unclear which sites in the iC3b and C3d molecules mediated the interaction. Certainly the sites are different from the metastable binding site of C3 (12), which attaches C3b covalently to acceptors on the *Candida* cell surface or to sensitized erythrocytes as used in the adherence assay.

Speculating on a possible meaning of this iC3b- and C3d-dependent effect in vivo, one is led by the restriction of interaction to highly pathogenic *Candida* species to suppose a role of iC3b or C3d binding in the pathogenetic mechanism. In human serum, *Candida* cells show a tendency to clump (3, 8; our own observations). This activity might be based on the formation of complement bridges between *Candida* cells that are opsonized (binding of C3 via acceptors) and those that have free binding sites for iC3b and C3d. Within such clumps, *Candida* cells are less accessible to phagocytes. Also, if the opsonins on their surface are occupied, iC3b-mediated phagocytosis would be impaired. This is reminiscent of the antiphagocytic effect of protein A of *Staphylococcus aureus*.

LITERATURE CITED

1. **Ballou, C. E.** 1976. Structure and biosynthesis of the mannan component of the yeast cell envelope. Adv. Microb. Physiol. **14**:93–158.
2. **Cabib, E., R. Roberts, and B. Bowers.** 1982. Synthesis of the yeast cell wall and its regulation. Annu. Rev. Biochem. **51**:763–793.
3. **Chilgren, R. A., R. Hong, and P. G. Quie.** 1968. Human serum interactions with Candida albicans. J. Immunol. **101**:128–132.
4. **Colten, H. R., T. Borsos, and H. J. Rapp.** 1969. Purification of the first component (C'1) of complement by zonal ultracentrifugation and the second (C'2) by electrofocusing. J. Immunol. **102**:1336.
5. **Dierich, M. P., H. H. Mussel, O. Scheiner, T. Ehlen, R. Burger, H. Peters, M. Schmitt, S. Trepke, and G. Zimmer.** 1982. Differentiation of C3b receptors on human lymphocytes, phagocytes, erythrocytes and renal glomerulus cells by monoclonal antibodies. Immunology **45**:85–96.
6. **Dierich, M. P., and T. F. Schulz.** 1984. Multifactorial basis of C3b receptor reactivity. Mol. Immunol. **12**:1201–1204.
7. **Farkaš, V.** 1979. Biosynthesis of cell walls of fungi. Microbiol. Rev. **43**:117–144.
8. **Hasenclever, H. F.** 1978. The in vitro interactions of Candida albicans with nonspecific serum proteins. Mycopathologia **65**:169–176.
9. **Hector, R. F., F. L. Lyon, and J. E. Domer.** 1981. Immunological relatedness among *Candida albicans* and other pathogenic *Candida* species. Infect. Immun. **34**:844–850.
10. **Kidby, D. K., and R. Davies.** 1970. Invertase and disulphide bridges in the yeast cell wall. J. Gen. Microbiol. **61**:327–333.
11. **Kornfeld, R., and S. Kornfeld.** 1976. Comparative aspects of glycoprotein structure. Annu. Rev. Biochem. **45**:217–237.
12. **Law, S. K., and R. P. Levine.** 1977. Interactions between the third complement protein and cell surface macromolecules. Proc. Natl. Acad. Sci. USA **74**:2701–2705.
13. **Mussel, H. H., T. Ehlen, M. Schmitt, M. E. Kazatchkine, L. Neyses, and M. P. Dierich.** 1982. Isolation and characterization of the C3b-binding entity of the C3b-receptor from human erythrocytes. Immunol. Lett. **4**:1–6.
14. **Schmitt, M., H. H. Mussel, and M. P. Dierich.** 1981. Qualitative and quantitative assessment of C3-receptor reactivities on lymphoid and phagocytic cells. J. Immunol. **126**:2042–2047.