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C3bi-Binding Protein on *Candida albicans*: Temperature-Dependent Expression and Relationship to Human Complement Receptor Type 3

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We investigated in detail the previously described capacity of pseudohyphae of *Candida albicans* to bind C3-coated particles. We show that the expression of the C3bi receptor of *C. albicans* was dependent upon the growth temperature of the fungi. *C. albicans* grown at 30°C bound strongly to EAC1423bi, whereas those cells grown at 38.5°C were completely devoid of this capacity. The molecule responsible for the attachment of EAC1423bi was heat labile and trypsin sensitive. Several, but not all, monoclonal antibodies to the α-chain of human complement receptor type 3 (CR3) stained *C. albicans*, and this reactivity was expressed in parallel with the capacity of *C. albicans* to bind EAC1423bi, i.e., both were dependent on the growth temperature of the fungi and were trypsin sensitive. In contrast to CR3, the binding of EAC1423bi to *C. albicans* did not require the presence of divalent cations. Rabbit immunoglobulin G antibodies directed against *C. albicans* inhibited the binding of EAC1423bi to *C. albicans* but not to human CR3. These inhibiting IgG antibodies recognized antigens expressed on the surface of pseudohyphae but not those of yeast cells. OKM-1, a monoclonal antibody to human CR3 inhibited the attachment of EAC1423bi to CR3 and also to *C. albicans*. OKM-1 precipitated a 130-kilodalton band from solubilized ^{125}I-labeled *C. albicans*. We conclude that the complement receptors on *C. albicans* and human CR3 were antigenically related but not identical and that they differed in their functional characteristics.

Various human cells express, on their surfaces, molecules which are able to bind fragments of complement component 3, like C3b, C3bi, and C3dg. Whereas C3b binds well to complement receptor type 1 (CR1) (1, 8), membrane cofactor protein (3), and decay-accelerating factor (18) and binds weakly to CR3, C3bi can be bound by CR3 (33), CR4 (23a) and, with lower affinity, can be bound by CR1 and CR2. C3dg and C3d both show a high affinity for CR2 (10, 32), and C3dg binds, although less strongly, to CR3. These C3-binding membrane molecules differ in their respective functions. CR1 is important for the binding and clearance of C3-coated immune complexes (20) and seems to contribute to the phagocytosis of some complement-coated microorganisms (34, 35). CR3 and CR4 are the most important receptors for the ingestion of complement-coated particles by phagocytes (31). Decay-accelerating factor seems to protect cells from an attack by homologous complement (21), and CR2 acts as a receptor for growth-promoting signals on B cells (9, 22, 24, 25).

C3-binding structures are not specific for human cells or mammalian cells in general but are also found in microorganisms pathogenic for humans. Mammalian cells infected with herpes simplex virus type 1 express, on their surface, glycoprotein C (gC) which is able to bind C3b (12). gC destabilizes in vitro the C3 convertase of the alternative pathway, suggesting that this gC might inhibit complement activation on the cell surface and thus protect the infected cell from complement-mediated lysis (13).

As we have originally shown (15) and as has been confirmed by others (7, 14), *Candida albicans* as well as *Candida stellatoidea* express C3bi- and C3dg-binding structures on their surfaces. The binding of the C3 fragments is restricted to the pseudohyphae of the dimorphic fungi. In addition, it could be demonstrated by immunofluorescence staining that two monoclonal antibodies to CR3, OKM-1 and Mo-1, reacted with *C. albicans* (7, 14). Details about biochemical and functional properties of the complement-binding structure on the surface of an opportunistic fungus are unknown. In this study, we performed further functional characterization of the C3bi-binding structure and investigated its relationship to CR3, the specific receptor for C3bi.

MATERIALS AND METHODS

Organisms and culture. *C. albicans* CBS 5982 (serotype A) was used throughout this study. After being grown on Sabouraud agar for 48 h at 37°C, the fungi was then stored at 4°C for 4 weeks. To transfer *C. albicans* into a dimorphic growth form, blastospores were inoculated from Sabouraud agar into RPMI 1640 (Flow Laboratories, Inc.) at a concentration of 10^5 cells per ml and incubated at 30, 37, and 38.5°C. Incubation of *C. albicans* blastospores in dextrose broth (Difco Laboratories) resulted in exclusive growth of yeast forms.

Monoclonal antibodies against complement receptors CR1, CR2, and CR3. Four monoclonal antibodies recognizing three different epitopes on CR2 were established in our laboratory (25). Monoclonal antibodies against CR3 were OKM-1 (Ortho Diagnostics) (33), MN-41 (6), Leu15 (Becton Dickinson and Co.) (26) (kindly provided by Louis Lanier), and M522 (29). MHM23 is a monoclonal antibody directed against the common β-chain of CR3, leukocyte function antigen-1 (LFA-1), and p150/95 (16). 3F11, an anti-CR1 monoclonal antibody, was also produced in our laboratory (28).

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Antiserum. New Zealand White rabbits were immunized by weekly subcutaneous injection of 10^8 CFU of viable *C. albicans* grown in RPMI 1640. Two weeks after the sixth immunization, rabbits were bled and the serum was stored at −80°C. Immunoglobulin G (IgG) was prepared from this serum by chromatography on QAE-Sephadex A 50 (Pharmacia).

EAC intermediates. Sheep erythrocytes were coated with complement components as described previously (5). Briefly, sensitized sheep erythrocytes were sequentially treated with purified human C1 (a kind gift of M. Loos, Institut fur medizinische Mikrobiologie, Mainz, Federal Republic of Germany) human serum, and oxidized human C2 (Cordis). These EAC142 were exposed to hemolytically active C3 at 37°C, thereby generating EAC1423b. Conversion to EAC1423bi was performed by incubation with heat-inactivated normal human serum dialyzed against Veronal-buffered saline containing saccharose (VBS-S) providing factors H and I. This incubation was carried out for 30 min at 37°C; under these conditions, 53% of bound C3b was converted to C3bi, as calculated by the amount of bound ^{125}I-labeled anti-C3c and ^{125}I-labeled anti-C3g (27).

Adherence assays. *C. albicans* CBS 5982 grown in RPMI 1640 for 20 h was washed with phosphate-buffered saline (PBS) and adjusted to 5×10^6 CFU/ml of PBS. Samples (20 μl) of this suspension were incubated with 20 μl of EAC1423bi adjusted to 1.3×10^8/ml of PBS. After the cells were incubated for 30 min at 37°C, the attachment index was determined by counting the number of adherent erythrocytes per 200 pseudohyphae and then dividing the resulting figure by 200 to obtain the average number of adherent erythrocytes per one pseudohypha. For inhibition assays 10-μl samples of washed fungi adjusted to 2.6×10^6/ml were preincubated with 10-μl samples of purified IgG at different dilutions for 30 min at 37°C and then reacted with 20 μl of EAC1423bi as described above.

To determine the binding of EAC1423bi to human CR3, cells of the human macrophage cell line U937 were stimulated with *O*-tetradecanoylphorbol 13-acetate (TPA) at a concentration of 15 ng/ml of RPMI. Samples (20 μl) of VBS-S-washed U937 adjusted to 1.3×10^6/ml were incubated with 20 μl of EAC1423bi at a concentration of 1.3×10^8/ml of VBS-S. After incubation for 30 min at 37°C, the percentage of rosette-forming cells was determined. Cells binding three or more sheep erythrocytes were counted as a rosette.

Preabsorption of rabbit IgG directed against *C. albicans* on *C. albicans*. To select antibodies which are expressed on the surface of pseudohyphae selectively, we preabsorbed rabbit IgG directed against *C. albicans* on *C. albicans* yeast forms and on *C. albicans* grown in RPMI forming about 10% pseudohyphae. The washed fungi were pelleted by centrifugation at $3,000 \times g$ and adjusted to a pelleted volume of 500 μl. Samples (500 μl) of rabbit IgG at concentrations of 60 and 300 μg/ml, respectively, were added and incubated at room temperature with constant shaking. After incubation, the washed fungi were pelleted by centrifugation and the supernatants were filtered through Millipore filters (0.2-μm pore size). The supernatants were tested for their inhibition capacity of the attachment of EAC1423bi on pseudohyphae as described above.

Trypsinization of *C. albicans*. Samples (10^7 CFU) of *C. albicans* suspended in 1 ml of RPMI 1640 were treated with trypsin (Sigma Chemical Co.) at concentrations ranging from 500 to 0.13 μg/ml of RPMI for 30 min at 37°C. Proteolysis was stopped by the addition of phenylmethylsulfonyl fluoride (Merck) to a final concentration of 2 mmol/liter. The reduction in the binding of EAC1423bi to trypsinized *C. albicans* was calculated by the following formula: [(attachment index of buffer-treated *C. albicans* cells − attachment index of trypsinized *C. albicans* cells)/(attachment index of buffer-treated *C. albicans* cells)] × 100.

Immunofluorescence assay. Samples (5×10^6 CFU) of PBS-washed *C. albicans* grown in RPMI 1640 were incubated with 100 μl of purified mouse IgG at a concentration of 120 μg/ml for 60 min at room temperature. Bound mouse IgG was detected with fluorescein isothiocyanate-conjugated goat IgG anti-mouse IgG (Tago). After each incubation step, the fungi were washed three times with PBS. The reaction was visualized with a Leitz Laborlux K microscope.

Immunoprecipitation of ^{125}I-labeled *C. albicans* proteins with anti-CR3 antibodies. A 0.5-ml sample of a PBS-washed *C. albicans* suspension adjusted to a concentration of 10^{10} CFU/ml was labeled with ^{125}I (Amersham Corp.) by the Iodo-Gen method as described previously (11). After being radiolabeled, proteins were extracted by mechanical treatment with a mortar and a pestle by the addition of nitrogen. The solubilized proteins were separated from nonsolubilized fragments by centrifugation at $10,000 \times g$ for 20 min. The supernatant was preabsorbed with bovine serum albumin coupled to CNBr-activated Sepharose (Pharmacia). Immunoprecipitation was carried out by OKM-1, M522, 2G7 (anti-CR2), and an anti-μ monoclonal antibody coupled to CNBr-activated Sepharose at a concentration of 1 mg of IgG per ml of Sepharose. Samples (30 μl) of each type of Sepharose were incubated with 170 μl of the ^{125}I-labeled protein solution for 1 h at room temperature. After the different Sepharose types were washed extensively with PBS, containing 600 mmol of NaCl per liter and 0.5% Nonidet P-40, they were solubilized in 50 mmol of Tris (pH 6.8) containing 2% sodium dodecyl sulfate (SDS), 5% mercaptoethanol, and 2% glycerol. Protein separation was performed by SDS-polyacrylamide gel electrophoresis (5 to 15% acrylamide) (19). To visualize the position of the precipitated proteins, the SDS gel was exposed on a Kodak X-ray film for 10 days.

RESULTS

Expression of a C3bi-binding structure on pseudohyphae of *C. albicans*: dependence on growth temperature. *C. albicans* inoculated in dextrose broth grew in their yeast form without forming pseudohyphae, whereas *C. albicans* inoculated in RPMI 1640 (without fetal calf serum) formed yeast cells as well as pseudohyphae. The morphology of the latter was dependent on growth temperature. When grown at 30 and 37°C, the fungi formed starlike pseudohyphae with highly elongated cells. Increasing the growth temperature to 38.5°C led to an increasing number of pseudohyphae. Under these conditions, the single cells were shorter than those formed at 30 and 37°C. To evaluate whether the changed morphology due to different growth temperatures had an influence on the ability of the pseudohyphae to bind EAC1423bi, *C. albicans* was grown in RPMI 1640 medium for 20 h at 30, 37, and 38.5°C. After the fungi were washed with PBS, the adherence assay with EAC1423bi was performed at 37°C for 30 min.

The attachment indices of the reaction between EAC1423bi and pseudohyphae grown at different temperatures were calculated. There was a marked difference in binding of the complement-coated erythrocytes to *C. albicans* grown at 30, 37, and 38.5°C, with maximal adherence of

TABLE 1. Reduction of EAC1423bi adherence to *C. albicans* CBS 5982 by heat pretreatment of the fungi[a]

Temp (°C)	Attachment index	% of EAC1423bi adherence[b]
30.0	3.30	100.0
38.5	3.20	97.0
50.0	3.20	97.0
56.0	0.45	13.5
60.0	0.13	3.9
65.0	0.04	1.2
70.0	0.00	0.0

[a] *C. albicans* CBS 5982 was grown in RPMI 1640 for 20 h at 30°C. Fungi were then subjected to elevated temperatures for 10 min before the adherence assay with EAC1423bi was performed as described in Materials and Methods. The values shown represent the mean of three determinations which varied by less than 10%.

[b] Compared with adherence of untreated controls.

EAC1423bi to the pseudohyphae formed at 30°C and nearly no binding of erythrocytes to pseudohyphae built at 38.5°C growth temperature. Specifically, the attachment indices for *C. albicans* CBS 5982 grown at the three temperatures, 30, 37, and 38.5°C, were 3.45, 1.45, and 0.17, respectively. The attachment index was the average of adherent EAC1423bi per pseudohypha. A total of 200 pseudohyphae were counted, and the total number of adherent EAC1423bi was divided by 200. The attachment indices given represent the means of three determinations which varied less than 10%. In all cases, the adherence was restricted to the pseudohyphae and did not occur on yeast forms.

As it is known that *C. albicans* synthesizes two heat stroke proteins (30 and 88 kilodaltons [kDa]) which are repressed at a temperature shift of 23 to 37°C within 60 min (4), we evaluated a possible temperature shift-dependent modulation of the expression of C3bi-binding structures on the surface of pseudohyphae. *C. albicans* grown at 30°C was subjected to 4 and 38.5°C for 2 h. Under these conditions, no change of the attachment indices could be detected.

Heat lability and trypsin sensitivity of EAC1423bi-binding structures on the surface of *C. albicans*. We could demonstrate that the C3bi-binding molecule expressed on the surface of *C. albicans* at a growth temperature of 30°C was heat labile. Submission of *C. albicans* (grown for 20 h at 30°C) to temperatures ranging from 30 to 70°C for 10 min led to an abrogation of EAC1423bi binding after exposure to a temperature of 56°C and higher values (Table 1).

To evaluate whether the C3bi-binding structure was trypsin sensitive, we exposed *C. albicans* grown in RPMI to trypsin at concentrations ranging from 500 to 0.13 μg/ml. After the cells were incubated for 30 min at 37°C, proteolysis was stopped with phenylmethylsulfonyl fluoride. The trypsin-treated fungi were washed extensively before adding the EAC1423bi. Figure 1 shows a concentration-dependent trypsin sensitivity of the C3bi-binding structures on the surface of *C. albicans*. There was a significant inhibition caused by trypsin concentrations up to 0.8 μg/ml, suggesting that the C3bi-binding structure on *C. albicans* has protein determinants.

Adherence of EAC1423bi to pseudohyphae of *C. albicans*: lack of dependence on divalent cations. Binding of C3bi to human and mouse CR3 requires Ca^{2+} and Mg^{2+}. To investigate whether the interaction between *C. albicans* and EAC1423bi would also be dependent on the presence of divalent cations, we performed the adherence assay with Ca^{2+}- and Mg^{2+}-free buffers containing 20 mmol EDTA.

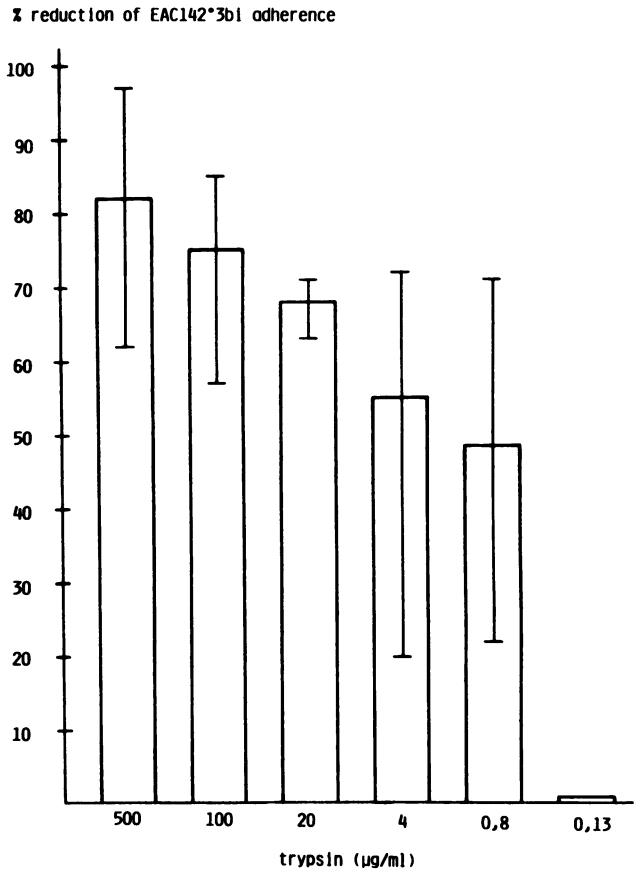

FIG. 1. Trypsin sensitivity of the *C. albicans* complement receptor. *C. albicans* grown at 30°C was pretreated with different concentrations of trypsin at 37°C for 30 min, and EAC1423bi binding was evaluated as described in Materials and Methods. Percent reduction of EAC1423bi binding to trypsinized *C. albicans* cells relative to buffer-treated *C. albicans* cells was calculated as described in Materials and Methods.

Under these conditions, the binding of EAC1423bi was the same as that in the presence of divalent cations.

Inhibition of C3bi binding to *C. albicans* by specific IgG antibodies. Preincubation of *C. albicans* pseudohyphae with native, as well as with heat-inactivated, normal human serum leads to an inhibition of EAC1423bi binding to the fungi. Because nearly all human sera contain antibodies to *C. albicans*, we investigated whether this phenomenon was due to IgG or to any other serum factors. Therefore, we preincubated the fungi with purified IgG from rabbits immunized with viable *C. albicans*. Figure 2 shows a concentration-dependent inhibition of EAC1423bi adherence to *Candida* cells by hyperimmune IgG, whereas IgG from nonimmune rabbits did not inhibit. Inhibition of EAC1423bi adherence was also exhibited by $F(ab')_2$ fragments of IgG at a concentration of 100 μg/ml (data not shown). Therefore, we suggest that the inhibition is due to immunoglobulins and that the molecules responsible for the complement attachment have immunogenic properties.

To test whether this inhibition is due to antibodies specific to pseudohyphae, we preabsorbed IgG on *C. albicans* yeast forms as well as on *C. albicans* pseudohyphae as described in Materials and Methods. Table 2 shows that IgG preabsorbed on yeast forms exhibited the same inhibition as that

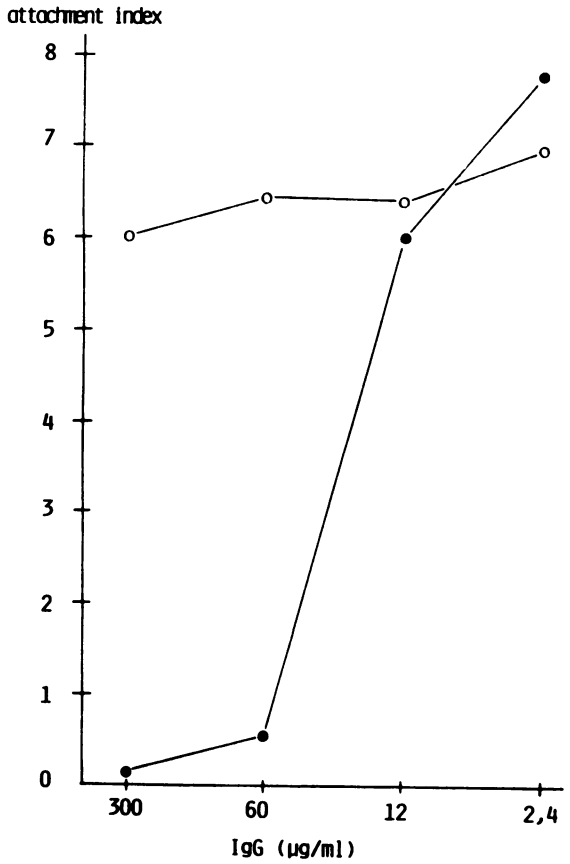

FIG. 2. Inhibition of EAC1423bi adherence to pseudohyphae by pretreatment with purified IgG from rabbits immunized with *C. albicans* (●) in comparison to pretreatment with IgG from nonimmunized rabbits (○).

of nonabsorbed IgG, whereas IgG preabsorbed on pseudohyphae had lost its inhibition capacity even at a concentration of 300 µg/ml. Immunofluorescence staining with the preabsorbed antibodies showed that there was no reactivity with antibodies preabsorbed on yeast cells and pseudohyphae, whereas antibodies preabsorbed on yeast cells alone conserved their ability to stain pseudohyphae.

Detailed analyses of the antigenic relationship of C3bi-binding structures on *C. albicans* with CR3. To further investigate the antigenic relationship between C3bi-binding

TABLE 2. Inhibition of EAC1423bi binding to *C. albicans* and immunofluorescence staining with preabsorbed rabbit IgG directed against *C. albicans*[a]

Preabsorption of rabbit IgG directed against *C. albicans*	% Inhibition of EAC1423bi binding	Immunofluorescence staining of:	
		Pseudohyphae	Yeast form
None (control)	98	++	++
Yeast forms	99	+	–
Yeast forms + pseudohyphae	0	–	–
	0[b]	–	–

[a] Preabsorption of specific IgG was carried out by incubating 500 µl of IgG at a concentration of 60 µg/ml of PBS with 500 µl of pelleted yeasts.

[b] In this experiment, the IgG concentration for preabsorption was elevated up to 300 µg/ml.

TABLE 3. Immunofluorescence staining of *C. albicans* with monoclonal antibodies directed against complement receptors[a]

Monoclonal antibody	Specificity for complement receptor	Immunofluorescent staining of:	
		Pseudohyphae	Yeast form
OKM-1	CR3 (α-chain)	+++	–
M522	CR3 (α-chain)	+	–
MN-41	CR3 (α-chain)	–	–
Leu15	CR3 (α-chain)	–	–
MHM23	CR3 (β-chain)	–	–
2G7	CR2	–	–
6F7	CR2	–	–
1C8	CR2	–	–
3F11	CR1	–	–

[a] CBS 5982 (grown in RPMI 1640 at 37°C) incubated with monoclonal antibodies were stained with fluorescein isothiocyanate-conjugated goat IgG against mouse IgG.

molecules on *C. albicans* and human CR3, we tested the reactivity of various monoclonal antibodies against both the α- and β-chains of CR3 by an immunofluorescence assay. For comparison, antibodies directed against CR1 (C3b receptor) and CR2 (C3dg receptor) were included. Table 3 shows that anti-CR1 and anti-CR2 antibodies failed to react. Among the anti-CR3 antibodies, OKM-1 and M522 demonstrated immunofluorescence staining of pseudohyphae but not of yeast forms of *C. albicans*. Two other monoclonal antibodies against the α-chain of CR3, Leu15 and MN-41, failed to react with *C. albicans*. Also, MHM23, an antibody directed against the common β-chain of CR3, CR4, and LFA-1, showed no immunofluorescence staining of the pseudohyphae. We investigated further whether the inhibiting polyvalent antibodies to *C. albicans* would compete with the binding of OKM-1. Preincubation of the fungi with rabbit IgG directed against *C. albicans* led to a mitigation of OKM-1 staining by immunofluorescence.

Since the binding of EAC1423bi to *C. albicans* was dependent on the growth temperature of the fungi, we investigated if OKM-1 binding would also vary on pseudohyphae grown at different temperatures. Therefore, *C. albicans* was grown at different temperatures as described above. According to the maximal adherence reaction of EAC1423bi, there was a maximal immunofluorescence staining of pseudohyphae grown at 30°C, with OKM-1. Markedly diminished, although not completely abolished, binding was seen on pseudohyphae which had been grown at 38.5°C. We could further demonstrate a mitigation of immunofluorescence staining by OKM-1 on trypsin-pretreated pseudohyphae of *C. albicans*, again indicating a parallel expression of the capacity to bind EAC1423bi and OKM-1. To investigate whether EAC1423bi and OKM-1 bind to the same or different areas on the surface of individual pseudohyphae, we performed the rosetting assay and immunofluorescence assay simultaneously (Fig. 3). Immunofluorescence staining with OKM-1, used in a subinhibitory concentration, showed that all pseudohyphae binding EAC1423bi also stained with OKM-1 whereas some pseudohyphae weakly reactive with OKM-1 did not bind the complement-coated sheep erythrocytes. We tested further whether antibodies directed against *C. albicans* which were shown to inhibit the EAC1423bi attachment on the fungi would cross-react with human CR3. By an immunofluorescence assay, we could not detect any reactivity of $F(ab')_2$ directed against *C. albicans* with CR3-expressing U937 cells.

Effect of OKM-1 and rabbit IgG directed against *C. albicans* on the binding of EAC1423bi to *C. albicans* and U937,

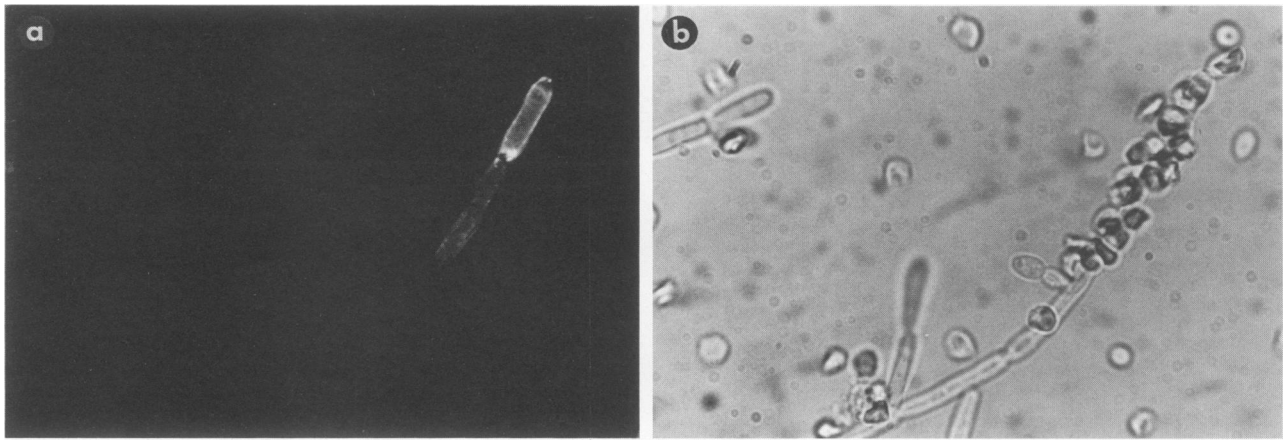

FIG. 3. Binding of EAC1423bi and immunofluorescence reactivity with OKM-1 are localized in the same area of an individual pseudohyphae. (a) Immunofluorescence staining with OKM-1 of a pseudohyphae. OKM-1 was used in a subinhibitory concentration (23 μg/ml). (b) Binding of EAC1423bi to a pseudohyphae.

respectively. To investigate whether the binding of OKM-1 to *C. albicans* would interfere with the attachment of EAC1423bi, we tested OKM-1 to see if it could inhibit the attachment of EAC1423bi to the pseudohyphae of the fungi. On the other hand, we investigated if antibodies directed against *C. albicans* which were seen to abolish the EAC1423bi binding to the fungi would also exhibit an inhibition of EAC1423bi binding to human CR3. Table 4 shows that the binding of EAC1423bi to *C. albicans* was inhibited by preincubation with OKM-1 at an IgG concentration of 300 μg/ml. A control antibody of the same isotype (IgG2b), directed against gC of herpes simplex virus type 1 did not inhibit. Preincubation of TPA-stimulated U937 cells with antibodies directed against *C. albicans* did not inhibit EAC1423bi binding to the cells, although OKM-1 led to a 75% inhibition of EAC1423bi rosettes.

Immunoprecipitation of ^{125}I-labeled surface molecules of *C. albicans* with OKM-1. OKM-1 can precipitate a noncovalently linked dimer of 165 and 95 kDa (α- and β-chains of CR3) from human macrophages. To determine which molecules on the surface of *C. albicans* would be recognized by this monoclonal antibody, we labeled *C. albicans* with ^{125}I. After *C. albicans* was radiolabeled, proteins were extracted by mechanical treatment of the fungi.

TABLE 4. Effect of OKM-1 and anti-*Candida* IgG on the binding of EAC1423bi to *C. albicans* and U937[a]

Cell and treatment	% Inhibition of EAC1423bi binding[b]
C. albicans CBS 5982 preincubated with:	
OKM-1 (300 μg/ml)	65.0 ± 14.6
OKM-1 (23 μg/ml)	8.7 ± 4.8
IgG2b[c] (300 μg/ml)	0.0 ± 0.0
TPA-stimulated U937 preincubated with:	
Rabbit IgG[d] (150 μg/ml)	5.7 ± 1.4
OKM-1 (12 μg/ml)	71.6 ± 3.3

[a] U937 cells were stimulated with TPA at a concentration of 15 ng/ml of RPMI. *C. albicans* and U937 were preincubated with the antibodies indicated, and rosette formation or EAC1423bi attachment was evaluated as described in Materials and Methods.
[b] Compared with binding of buffer-treated control.
[c] Monoclonal antibody directed against gC of herpes simplex virus type 1.
[d] Rabbit IgG directed against *C. albicans*.

Immunoprecipitation was carried out by OKM-1 and M522 coupled to CNBr-activated Sepharose. We used 2G7 (anti-CR2) and an anti-μ monoclonal antibody for controls. Figure 4 shows that OKM-1 specifically precipitated a molecule with a molecular size of 130 kDa. In addition, two minor bands could be observed corresponding to 50 and 100 kDa. A band at approximately 500 kDa was also apparent in the material precipitated by bovine serum albumin-Sepha-

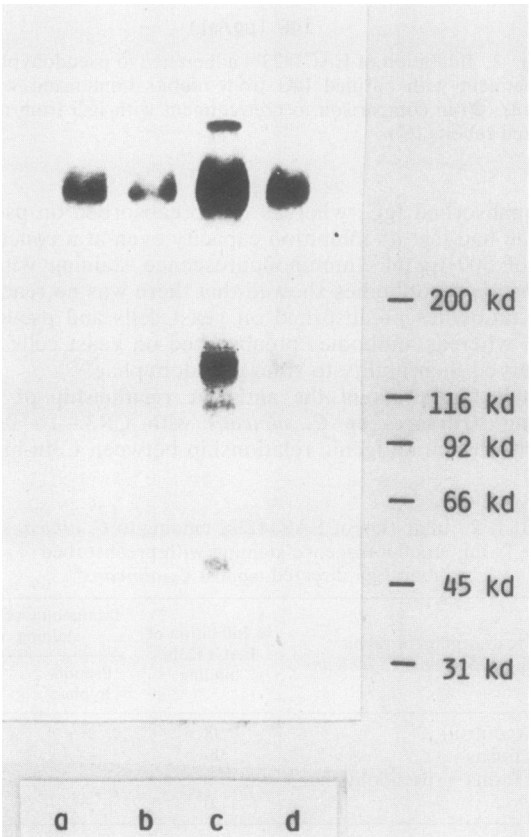

FIG. 4. Immunoprecipitation of ^{125}I-labeled *C. albicans* with OKM-1 (lane c) and M522 (lane b). Control antibodies were 2G7 (anti-CR2) (lane a) and an anti-μ antibody (lane d). kd, Kilodaltons.

rose as well as by 2G7-, anti-μ, and M522-Sepharose; it probably represented iodinated material nonspecifically adhering. The estimation of the high-molecular-size band was carried out by running the preparation on a SDS-polyacrylamide gel (4.5% acrylamide) with low-density lipoprotein as a marker for a 500-kDa protein.

DISCUSSION

In this study, we attempted a further functional characterization of the structure on *C. albicans* mediating the attachment of EAC142bi and investigated its relationship to human CR3. We show that the capacity of *C. albicans* to bind EAC1423bi was dependent on the growth temperature of the fungi. The maximal expression of the molecule at 30°C was reduced about 50% at 37°C and was completely abolished by increasing the temperature to only 38.5°C. In addition, we demonstrate that the C3bi-binding structure on *C. albicans* was heat labile and sensitive to trypsin and therefore most probably a protein. Furthermore, we report the reactivity of *C. albicans* with another monoclonal antibody to human CR3, M-522, which like OKM-1 (14) and Mo-1 (7, 14), reacts with the α-chain of CR3. As we could show that both the reactivity of OKM-1 with *C. albicans* and the binding of EAC1423bi to *C. albicans* were growth temperature dependent and trypsin sensitive, and as EAC1423bi bound to those regions of the pseudohyphae that also stained with OKM-1, we conclude that the molecules on the surface of *C. albicans* mediating the C3bi- attachment and the OKM-1 binding were probably identical. The inhibition of EAC1423bi binding to *C. albicans* by OKM-1 supports this suggestion. This inhibition was effective only at a relatively high concentration of the antibody. The fact that some areas on the pseudohyphae stained with OKM-1 but did not bind EAC1423bi could be explained by a possible steric hindrance of EAC1423bi binding or by a possible difference in the receptor density required for OKM-1 and EAC1423bi attachment. However the C3bi-binding molecule on *C. albicans* is not identical with human CR3, because Leu15 and MN-41 as well as an anti-β-chain monoclonal antibody did not bind to the surface of the fungi. In addition, immunoprecipitation of ^{125}I-labeled *C. albicans* cells with anti-CR3 antibodies did not reveal a dimmer of 165 and 95 kDa. Instead, a molecule of 130 kDa was precipitated from iodinated membrane material of *C. albicans* cells, concomitant with some material of 50, 100, and 500 kDa, with the latter being also observed in controls.

There were also functional differences between CR3 and the C3bi-binding protein on *C. albicans*. In contrast to C3bi binding to human CR3, the attachment of C3bi to *C. albicans* did not require the presence of divalent cations. IgG antibodies directed against *C. albicans* which abolished C3bi binding to the fungi did not inhibit C3bi binding to CR3 (Table 3).

Various human cells express, on their surface, receptors for extracellular glycoproteins. This family of receptors includes the receptor for fibronectin, vitronectin, laminin, and also C3bi (CR3 and CR4). Each of them consists of two noncovalently linked subunits, a 95- to 130-kDa protein (β-chain) and a 130- to 210-kDa protein (α-chain). The receptors show homologies in their β-chains and most of them, including CR3, contain in their α-chain a binding site for the amino acid sequence RGD (Arg-Gly-Asp) which is involved in ligand binding (17). As it is known that *C. albicans* can bind to fibronectin (30) and as we have shown that the C3bi receptor on *C. albicans* has an antigenic relationship to CR3, it will be interesting to investigate if there are similarities between surface molecules of *C. albicans* and integrins on human cells which are responsible for cell-cell as well as cell-matrix interactions. Maybe there are more members of attachment molecules on the surface of *C. albicans* which may have been partially conserved throughout evolution. Preliminary results indicate that *C. albicans* can bind a peptide containing the amino acid sequence RGD and that this binding interferes with the C3bi binding on the pseudohyphae (data not shown). The 130-kDa molecular entity precipitated from *C. albicans* by an antibody against CR3 (OKM-1) and the inhibition effect of the RGD peptide on C3bi attachment to *C. albicans* would be compatible with the concept that the C3bi-binding protein on *C. albicans* is a member of the integrin family (5a). The variability of the expression of the C3bi receptor on *C. albicans* being dependent on the growth temperature is very interesting with respect to the possibility that this molecule could be a pathogenic factor of *C. albicans*, e.g., mediating adherence to cell surfaces.

It has previously been reported by Calderone et al. that there are two proteins (62 and 70 kDa) on the surface of *C. albicans* which act as C3d receptors (2). Whether these two molecules have the functional characteristics reported here and are antigenically related to human CR3 will have to be investigated.

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