Mouse Sialoadhesin Is Not Responsible for *Candida albicans* Yeast Cell Binding to Splenic Marginal Zone Macrophages

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*Candida albicans* hydrophilic yeast cells specifically adhere to mouse macrophages in the splenic marginal zone and in lymph node subcapsular and medullary sinuses. These macrophages express sialoadhesin that binds erythrocytes, but binding of yeast cells is not mediated by sialoadhesin because (i) erythrocytes did not block yeast cell binding, (ii) yeast cell adherence was unaffected by sialoadhesin-specific monoclonal antibodies, (iii) the purified sialoadhesin did not bind to yeast cells, and (iv) an Fc-sialoadhesin chimera immobilized on plastic did not bind yeast cells. These data give further support for a unique macrophage adhesion system that binds *C. albicans*.

Adherence properties of *Candida albicans* are critical in colonization of the host and may also be important in pathogenesis of candidiasis (1, 4). We previously reported that *C. albicans* yeast cells bind selectively to macrophages within the marginal zone of the spleen (9) and in the subcapsular and medullary sinuses of peripheral lymph nodes (6). The adhesion system expressed by these macrophages appears to be unique (6, 9). Recently, van den Berg et al. showed that sialoadhesin expressed on murine tissue macrophages binds human erythrocytes and also functions as a lymphocyte adhesion molecule (13). The tissue binding distribution pattern of a TK-1 lymphoma cell line (13) shows a striking similarity to the binding pattern of *C. albicans* yeast cells in both spleen and lymph node tissues (5, 6, 8). Furthermore, sialoadhesin is a mouse macrophage-specific cell surface receptor (3), and its expression is restricted to splenic marginal zone and lymph node subcapsular and medullary sinus macrophages (2). In the present study, use of an ex vivo adherence assay (4, 8, 11) and binding assays with purified sialoadhesin showed that sialoadhesin does not mediate yeast cell binding to splenic marginal zone macrophages.

*C. albicans* A-9 (serotype B) and Ca-1 (serotype A) were grown and used as hydrophilic yeast cells as described previously (5, 7, 9, 10). Human blood was obtained from a healthy volunteer, and a TK-1 lymphoma cell line was a gift from Mark A. Jutila, Montana State University. Splenic tissues were obtained from 8- to 10-week-old male BALB/cByJ and [BALB/cByJ × Cr1:CD-1(1CR)BR]/F1 mice. A *C. albicans* yeast cell adhesion fraction (2-mercaptoethanol extract fraction II [2-ME extract Fr.II]) that blocks yeast cell binding to spleen and lymph node tissues was prepared as described previously (8). The rat antismouse monoclonal antibodies (MAbs) used in this study were SER-4, 3D6 [immunglobulins G (IgG) and their F(ab')2 fragments], and 2E8 (IgG), each of which recognizes a distinct epitope on the sialoadhesin molecule (references 2 and 13 and unpublished observations). For use in the ex vivo adherence assay, each of the antibodies was suspended to a concentration of 10 μg of antibody protein per ml of RPMI 1640 medium containing 25 mM N-2-hydroxethyl-piperazine-N’-2-ethanesulfonic acid (HEPES; GIBCO, Grand Island, N.Y.) and 5% fetal bovine serum (HyClone Laboratories Inc., Logan, Utah) and adjusted to pH 7.4. The complete and pH-adjusted medium is referred to as RPMI.

The ex vivo assay was performed as described previously for *C. albicans* yeast cells (9, 11). In this study, either *C. albicans* yeast cells or erythrocytes, each at 1.5 × 106 cells per ml of the described cell culture medium (9, 11), were assayed for adherence. In some experiments, a mixture of equal amounts of yeast cells and erythrocytes was used. In other experiments, cryosections of spleen were pretreated with the 2-ME extract Fr.II (10 μg/ml; i.e., the solubilized adhesins from *C. albicans*) as described previously (8) prior to the addition of the yeast-erythrocyte cell mixture.

In ex vivo assays that involved the antibodies, spleen cryosections were pretreated at 4°C for 1 h with 100 μl of a pool either of MAbs SER-4, 3D6, and 2E8 or of F(ab')2 fragments of SER-4 and 3D6. Most of the unbound antibodies were decanted from the tissue sections, 100 μl of the yeast suspension (1.5 × 106 cells per ml) was added, and yeast cell adherence to the splenic marginal zone was quantified (8, 9, 11). Positive adherence control tissues were pretreated with RPMI only. Activity of the MAbs and their F(ab')2 fragments was shown by their ability to block adherence of the TK-1 lymphoma cells as described before (13), with two minor modifications. Pretreatment of tissues with each pool of the MAbs and their fractions was done at 4°C for 1 h, and 0.1% thionin (Sigma Chemical Co., St. Louis, Mo.) staining solution was dissolved in Dulbecco’s phosphate-buffered saline (PBS [Sigma]; pH 7.4) before use.

Direct binding assays with radiolabeled sialoadhesin to *C. albicans* yeast cells were performed. Sialoadhesin was purified from mouse spleen and radioiodinated as described previously (3). For binding assays with the radiolabeled receptor, cells were incubated with a total of 80 fmol of 125I-sialoadhesin in 50 μl of 1.0% bovine serum albumin in PBS (BSA-PBS) for 1 h at room temperature and washed, and the bound radioactivity...
was determined as described previously (13). For antibody inhibition controls, 125I-sialoadhesin was preincubated with 40 μg of 3D6 F(ab')2 per ml for 1 h at room temperature before the addition of cells.

In some experiments, the ability of radioiodinated C. albicans yeast cells to bind to an Fc-sialoadhesin chimera fixed to a microtiter plate was measured. The results were compared with the binding of radioiodinated human erythrocytes to the same chimera preparation. This Fc-sialoadhesin chimera was prepared as follows. A 5.8-kb cDNA encoding the complete open reading frame of sialoadhesin was cloned into pBlueScript II (Stratagene, La Jolla, Calif.). Oligonucleotide primers designed to be compatible with the pIG plasmid, which contains genomic DNA encoding the hinge, CH2, and CH3 domains of human IgG1 (12), were used to amplify a 0.7-kb fragment that encodes the extracellular portion which is adjacent to the predicted transmembrane region. This was ligated to the remaining 5' sequence encoding the rest of the extracellular domain. An EcoRI fragment encoding the entire extracellular domain was ligated into the EcoRI site of pIG (12) (kindly provided by D. L. Simmons, ICRF Labs, Institute of Molecular Medicine, Oxford, United Kingdom) to produce the plasmid pIGSn1. COS-7 cells were transiently transfected by the DEAE-dextran method with pIGSn1 as described previously (12), and supernatants were harvested after 8 days. The Fc-sialoadhesin chimera was purified by affinity chromatography with protein A-Sepharose (Pharmacia, Uppsala, Sweden). The eluates were dialyzed into 20 mM Tris (pH 8.0) and concentrated to 1.0 mg/ml. Yeast cells and erythrocytes were radioiodinated by the Iodogen (Pierce Chemical Co., Rockford, Ill.) method as follows. A 100-μl cell suspension in PBS containing 2 × 10^8 C. albicans yeast cells or 1 × 10^7 erythrocytes was added to a microcentrifuge tube precoated with 5 μg of Iodogen. The addition of 2.4 MBq of Na125I (Amersham, Arlington Heights, Ill.) followed. After 15 min of incubation at room temperature, the reaction was stopped by transferring the cell suspension to a fresh microcentrifuge tube.

### Table 1. Binding of sialoadhesin to human erythrocytes and C. albicans yeast cells

<table>
<thead>
<tr>
<th>Type of cell (no. of cells interacting with sialoadhesin)</th>
<th>Amt bound ± SEM (fmol)</th>
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<tbody>
<tr>
<td></td>
<td>125I-Sialoadhesin</td>
</tr>
<tr>
<td></td>
<td>125I-Sialoadhesin + 3D6 F(ab')2</td>
</tr>
<tr>
<td>Erythrocytes (2.5 × 10^7)</td>
<td>19.7 ± 1.5</td>
</tr>
<tr>
<td>C. albicans Ca-1 (2.2 × 10^7)</td>
<td>0.52 ± 0.08</td>
</tr>
<tr>
<td>C. albicans A-9 (2.2 × 10^7)</td>
<td>0.32 ± 0.08</td>
</tr>
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</table>

FIG. 1. Adherence of C. albicans yeast cells and erythrocytes to the marginal zone of mouse spleen. Cryosections of spleen from [BALB/cByJ × CrI:CD-1(ICR)BR/F] mice were allowed to interact with either medium only (A), 125I-sialoadhesin (B), or 2 ME extract Fr.II (C), and then 100 μl of yeast-erythrocyte cell mixture (0.1 ml of a concentration of 1.5 × 10^9 of each cell type per ml) was added and the mixture was incubated for 15 min at 4°C. The tissues were stained with crystal violet which preferentially stained the yeast cells (arrowheads), leaving the larger erythrocytes unstained (arrows). Note that in panel B, only one erythrocyte (arrow) is found compared with many yeast cells (arrowheads), whereas in panel C, only one yeast cell (arrowhead) is found compared with many erythrocytes (arrows). W, white pulp; R, red pulp; M, marginal zone. Bar, 20 μm.
containing 1 ml of BSA-PBS. The radiolabeled cells were washed extensively with BSA-PBS. The specific activities of radiolabeling ranged from 200 to 300 cpm/10^3 C. albicans yeast cells and 80 to 100 cpm/10^3 erythrocytes. For binding assays with the Fc-sialoadhesin chimera, wells of microtiter plates (Greiner, Alphen a/d, The Netherlands) were coated with 100 μl of anti-human IgG antibody (15 μg/ml; Sigma) suspended in bicarbonate buffer solution at pH 9.5, incubated at 4°C for overnight, washed with the bicarbonate buffer solution, and blocked with 5% skim milk for 1 h at 37°C before use. The Fc-sialoadhesin chimera (10 μg/ml) prepared in BSA-PBS was placed into each well and incubated for 2 h at 37°C. Labeled cells (i.e., yeast cells or erythrocytes) were added to the wells and allowed to interact with the chimera for 1 h at room temperature. After three washes in BSA-PBS, cell-bound radioactivity was extracted with 1 N NaOH for 30 min, and the counts per minute of the extract was determined as described previously (13). As a control, cells were added to wells without the Fc-sialoadhesin chimera. In other experiments, cells were treated with sialidase as described before (5) prior to being added to the Fc-sialoadhesin-adsorbed wells.

When yeast cells were mixed with human erythrocytes, both cell types bound to the splenic marginal zone (Fig. 1A) as expected (2, 5). However, the location of binding within the marginal zone differed slightly for the two cell types. Yeast cells generally bound to the red pulp side of the marginal zone, whereas erythrocytes bound in the marginal zone closest to, and extending into, the white pulp. Since erythrocytes bind to macrophages via sialoadhesin (2), these observations suggested that C. albicans yeast cell binding is mediated by a different macrophage adhesion system and may involve a different subpopulation of macrophages within the marginal zone. Furthermore, tissues pretreated with SER-4, 3D6, or a pool of their F(ab')2 fragments did not bind TK-1 cells but bound yeast cells normally (data not shown). Likewise, tissues pretreated with SER-4 MAb essentially lost their ability to bind erythrocytes but not yeast cells (Fig. 1B). Conversely, tissues pretreated with the 2-ME extract Fr.II lost the ability to bind yeast cells but not erythrocytes (Fig. 1C).

Although these results strongly support the conclusion that the macrophage sialoadhesin does not bind yeast cells, the possibility remained that the MAb do not block the yeast adherence site on the sialoadhesin molecule. Purified sialoadhesin was thus tested for its ability to directly bind to yeast cells. Whereas 125I-sialoadhesin bound to human erythrocytes, the labeled adhesin showed virtually no evidence of binding to yeast cells (Table 1). In titration experiments not shown, as few as 2,000 erythrocytes bound more sialoadhesin than 2.2 x 10^7 yeast cells did. Furthermore, the relatively small amount of sialoadhesin binding to yeast cells was not affected by sialoadhesin-specific antibody (Table 1).

It may be hypothesized that only immobilized sialoadhesin binds to yeast cells. This hypothesis was tested by constructing an Fc-sialoadhesin chimera to allow attachment of the complex to anti-Fc-coated microtiter plates. The solid-phase binding assay showed that the number of yeast cells binding to coated wells was not different from the number of yeast cells that bound to uncoated control wells (Table 2). In positive controls, human erythrocytes bound to uncoated wells, and sialidase treatment of the erythrocytes abolished their ability to bind to coated wells.

The data presented in this study indicate that binding of hydrophilic C. albicans yeast cells to splenic marginal zone macrophages does not involve sialoadhesin. In previous work, other known host adhesin systems were also found not to be responsible for yeast cell binding (6, 9). The identification of the macrophage receptor for yeast cells is under investigation.

We thank Soo-kyung Han for assistance in performing some of the ex vivo assays. This work was supported by a collaborative research grant from NATO and by grant AI24912 from the National Institutes of Health.

### TABLE 2. Binding of human erythrocytes and C. albicans yeast cells to the immobilized Fc-sialoadhesin chimera

<table>
<thead>
<tr>
<th>Type of cell added to each well (no. of cells)</th>
<th>Wells coated with the Fc-sialoadhesin chimera</th>
<th>Uncoupled control wells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocytes (1.0 x 10^9)</td>
<td>(13.6 x 10^6) ± (0.3 x 10^6)</td>
<td>(0.1 x 10^6) ± (0.01 x 10^6)</td>
</tr>
<tr>
<td>Sialidase-treated erythrocytes (1.0 x 10^9)</td>
<td>(0.2 x 10^6) ± (0.01 x 10^6)</td>
<td>(0.39 x 10^6) ± (0.12 x 10^6)</td>
</tr>
<tr>
<td>Yeast cells, strain Ca-1 (7.0 x 10^5)</td>
<td>(1.5 x 10^6) ± (0.03 x 10^6)</td>
<td>(3.1 x 10^6) ± (1.1 x 10^6)</td>
</tr>
</tbody>
</table>

* a Estimate of total number of cells bound per well equals total counts per minute per well divided by counts per minute per cell; variance is expressed as the standard error of the mean.

### REFERENCES

