Specific Binding of Human Fibrinogen Fragment D to Aspergillus fumigatus Conidia

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The interaction of purified human fibrinogen with Aspergillus fumigatus conidia was investigated by immunofluorescence and electron microscopy and binding assays with radiolabeled proteins. We described the localization of the binding sites on the A. fumigatus conidia and on the fibrinogen molecule and determined the binding characteristics. Immunofluorescence revealed that the fixation of purified fibrinogen was selectively associated with conidia and suggested a role for the D domains of the fibrinogen molecule. Binding assays performed with 125I-radiolabeled proteins confirmed that binding sites were located specifically in the D domains. No reaction could be detected with fragment E. The binding of 125I-fragment D to conidia was time dependent, saturable, and specific. Scatchard analysis of the data revealed an average of 1,200 binding sites per conidium, and an apparent dissociation constant (Kd) of 2.2 × 10^-9 M was estimated. Pretreatment of the cells with proteolytic enzymes or heat abolished binding, demonstrating the protein nature of the binding sites. Ultrastructural localization of the fungal receptors was determined by transmission electron microscopy. Labeling appeared to be associated with the outer electron-dense layer of the conidial wall and progressively decreased during the germination process. Labeling of thin sections with fragment D and an antifibrinogen immune serum revealed that binding sites also lay in the inner part of the wall and in vacuoles. These results indicate the presence at the conidial surface of specific receptors for fibrinogen which could act as mediators of conidial adherence to host tissues.

Fungi of the genus Aspergillus are responsible for various infections, such as aspergiloma, bronchopulmonary aspergillosis, and invasive aspergillosis (5, 35). These primarily respiratory pathogens mainly infect immunocompromised hosts, i.e., patients with a functional deficiency in their neutrophils, like that seen with chronic granulomatous disease, or patients with prolonged neutropenia induced by cytotoxic drugs (15). These infections are often fatal, and their prevalence has markedly increased during the last few years with the development of immunosuppressive therapy (4, 14).

Elucidation of the host defense mechanisms in aspergillosis has been the focus of many scientific investigations in recent years. However, the molecular basis of the pathogenesis of these fungi remains to be determined. Initial interactions with the host cells and colonization of mucosal surfaces are now considered crucial steps in the establishment of infections. Ligands involved in adherence have been identified for various microorganisms. Among them, fibrinogen, which plays a key role in inflammatory reactions and in the coagulation pathway, seems to be a good candidate for mediating adherence. The involvement of such an interaction has been emphasized for different bacteria, such as Staphylococcus aureus (16, 39), streptococci of groups A, C, and G (23, 24, 42) and B (25), Bacteroides gingivalis (26, 27), and B. intermedius (26, 28), as well as for the opportunistic fungus Candida albicans (1, 6, 41). The binding of fibrinogen to microorganism cell walls could also inhibit the binding of complement and therefore opsonization and phagocytosis, as demonstrated for group A, B, C, and G streptococci (8, 43). However, little information is available concerning the structures responsible for these interactions. Fibrinogen, which is a dimer of three nonidentical polypeptide chains, designated Aα, Bβ, and γ and linked by disulfide bonds, is converted after plasmin cleavage into one E fragment and two D fragments (31). The former constitutes the central nodule (core) of the molecule, and the latter correspond to identical outer domains. Hawiger et al. (16, 39) have demonstrated that the binding site of the staphylococcal clumping factor is localized on the carboxy-terminal segment of the human fibrinogen γ chain. In contrast, fibrinogen fragment D binds specifically to C. albicans (1) and to the M protein of Streptococcus pyogenes (38), whereas B. gingivalis is linked to the intact γ chain but not to fragment D or E (26).

We previously demonstrated that, among filamentous fungi belonging to different groups (opportunistic fungi, strictly saprophytic or phytopathogenic fungi, and dermatophytes or related species), only the pathogenic aspergilli, particularly Aspergillus fumigatus, the main causative agent of aspergillosis in humans, bind crude fibrinogen (7). The binding was detected mainly at the surface of the conidia, which represent the dissemination form of the fungus in the environment. The aim of the present study was to identify the binding site on the fibrinogen molecule and to determine the binding characteristics. We also attempted to characterize the fibrinogen receptors on A. fumigatus conidia.

MATERIALS AND METHODS

Organisms and growth conditions. A. fumigatus 113.26, obtained from the Centraalbureau voor Schimmelcultures (Baarn, The Netherlands), was cultured on Sabouraud dex-
trose agar (Difco Laboratories, Detroit, Mich.). After incubation for 5 days at 37°C, fungal suspensions were prepared by flooding the agar plates with approximately 10 ml of sterile distilled water and scraping the aerial mycelium (hyphae and conidia). The suspensions were filtered through a 25-μm-pore-size nylon MOnyl membrane (Sarstedt, NY) or Zürcher Beuteltuchfabrik A.G., Rüschlikon, Switzerland) to remove the possible bits of agar, the conidial heads, and the hyphal fragments. The conidial suspensions were finally centrifuged (1,200 g; pH 7.2). Purified conidia were then inoculated into 10 ml of sterile medium (saccharose, 30 g; NaNO₃, 3.0 g; KH₂PO₄, 1.0 g; MgSO₄, 0.5 g; KCl, 0.5 g; FeSO₄·7H₂O, 0.01 g; chloramphenicol, 1.0 g; distilled water, 1,000 ml). Incubation was carried out in polystyrene petri dishes (each containing 15 ml of culture medium) until the spores germinated (about 12 h at 37°C). Finally, fungal elements were harvested by centrifugation and washed twice in phosphate-buffered saline (PBS; 0.15 M; pH 7.2).

**Fibrinogen and related proteins.** Human fibrinogen (Kabi Diagnostica, Stockholm, Sweden), which contains a small amount of other adhesive proteins, such as fibroenectin, was purified by serial affinity chromatography on gelatin-Ultrogel (13) and heparin-Ultrogel A4R (12) (Pharmacia-LKB, Uppsala, Sweden) as previously described (1). Analysis of the obtained fibrinogen by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing and nonreducing conditions revealed the expected bands after Coomassie blue staining; no significant contamination was seen. Moreover, fibrinogen was shown to be free of fibroenectin by immunoblotting. Aliquots of the fibrinogen solution were stored at −80°C until use. Fragments D and E of fibrinogen, obtained by plasmic digestion, were a gift from Diagnostica Stago (Asnières, France).

**Iodination of proteins.** Fibrinogen and its fragments D and E were labeled with 125I (IMS 30; Amersham, Buckinghamshire, United Kingdom) by the chloramine-T method (18). Labeled proteins were separated from free iodine by gel permeation through Sephadex G-25M columns (PD 10; Pharmacia-LKB) previously equilibrated with PBS supplemented with bovine serum albumin (BSA; 1 mg/ml). The specific activities of radiolabeled fibrinogen, fragment D, and fragment E were 0.72, 0.84, and 1.85 mCi/mg, respectively.

**Immunofluorescence assay.** The conidial suspensions (10⁶ conidia per tube) were centrifuged for 5 min at 3,000 × g, and the pellets were resuspended in 200 μl of PBS containing 0.1% sodium azide, 0.5% sodium citrate, and 1% BSA (BSA-PBS). Purified fibrinogen, fragment D, or fragment E was added at a 0.5-mg/ml final concentration. After incubation for 30 min at 37°C with constant shaking, the conidia were washed three times in PBS and incubated for 30 min at 37°C in 200 μl of rabbit anti-human fibrinogen immune serum (Diagnostica Stago) at 1:50 dilution in BSA-PBS. After several washings, the cells were incubated for 30 min at 37°C in 200 μl of fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulin G antibodies (Biosys, Compiègne, France) at a 1:100 dilution in BSA-PBS. Finally, the conidial suspensions were washed three times in PBS, dropped onto glass slides, mounted in glycerol-PBS (9:1 [vol/vol]), and examined under a Nikon microscope equipped for epifluorescence. Control experiments were performed by omitting plasma proteins or by using rabbit anti-human fibroenectin immune serum (at a 1:50 dilution in BSA-PBS; Sigma Chemical Co., St. Louis, Mo.) instead of rabbit anti-human fibrinogen immune serum.

**Assay for binding of radiolabeled proteins to conidia.** Quantification of binding was carried out as described by Hawiger et al. (16). Binding assays were performed with polystyrene tubes previously saturated by overnight incubation at 4°C with 1% BSA in PBS to minimize nonspecific binding of proteins and cells to the tube surface. Unless otherwise stated, aliquots of the conidial suspensions (10⁶ cells) were incubated with 125I-radiolabeled proteins, fibrinogen, and its D or E fragment (5 × 10⁻¹² mol in PBS containing 0.1% sodium azide and 0.5% sodium citrate [PBS-C] per assay) in a final volume of 200 μl. Then, 150 μl of BSA-PBS or inhibiting compound solutions was added to the tubes. After 90 min of shaking at 37°C, duplicate 150-μl samples of the incubation mixture were applied to a cushion of 200 μl of dibutylphthalate-paraffin oil (9:1 [vol/vol]) in conical Eppendorf tubes and centrifuged immediately (12,000 × g for 3 min). The contents of the tubes were frozen in liquid nitrogen. The tips of the tubes, containing the pellets, were separated from the tops, corresponding to unbound ligand, with a cutter plier. The radioactivity of the contents was finally assayed with a gamma counter (Crystal 5400; Packard Instrument Co., Downers Grove, Ill.). Saturability was studied with various amounts of radiolabeled fragment D, ranging from 0.5 to 6 μg in a final volume of 350 μl. Competitive binding was determined in the presence of a 100-fold molar excess of unlabeled ligand. Specific binding was ascertained by subtracting nonspecific binding from total binding. All experiments were carried out in duplicate, and results were analyzed by Scatchard plot analysis (37).

**Heat and enzyme pretreatments of conidia.** The effect of heat pretreatment was investigated by incubation of the conidial suspensions at 56, 80, or 95°C for different times (5 to 60 min) in a water bath. The suspensions were then cooled on ice and used for the binding assay.

In other experiments, the conidial suspensions were incubated with increasing amounts (100 to 1,000 μg/ml) of trypsin or chymotrypsin for 30 min at 37°C. The reactions were stopped by the addition of phenylmethylsulfonyl fluoride (PMSF; 0.1 M solution in methanol) at a final concentration of 1 mM. The cells were then centrifuged, washed twice with PBS, and used for binding studies.

**Immuno-electron microscopy.** The binding sites were localized by electron microscopy. Two different methods were used for fungal suspensions containing all evolutionary stages from resting conidia to germ tubes: (i) **Immuno-electron microscopy before embedding.** Cells were incubated for 1 h at 20°C with 0.5 ml of human fibrinogen fragment D solution (0.5 mg/ml in PBS). They were then washed in PBS and treated with 0.5 ml of rabbit anti-human fibrinogen at a 1:50 dilution in PBS containing 0.1% BSA for 1 h. After several washings in PBS–0.1% BSA, protein A-gold (1:10 dilution in PBS–0.1% BSA) was added to the samples and incubated for 30 min. Fixation was done with 2.5% glutaraldehyde buffered at pH 7.2 with 0.1 M sodium cacodylate for 1 h. Cells were postfixed for 30 min in 1% OsO₄ buffered with 0.1 M sodium cacodylate (pH 7.2), dehydrated in ethanol, and embedded in Epon. (ii) **Immuno-electron microscopy after embedding.** Cells were fixed for 1 h at 20°C in a mixture consisting of 2% freshly prepared formaldehyde and 0.2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2). After being washed in cacodylate buffer, the samples were treated for 30 min with 50 mM ammonium chloride prepared in the same buffer, washed again, and treated for 30 min at 20°C with a 0.5% solution of
uranyl acetate (2). Dehydration and embedding with Lowicryl K4M (Chemische Werke Lowi, Waldkraiburg, Germany) at progressively lower temperatures were done as previously described (36). In brief, the samples were dehydrated with 70% ethanol for 30 min at 4°C, with 95% ethanol for 1 h at -18°C, and twice with 100% ethanol at -18°C for 1 h each time. Dehydration was followed by treatment with mixtures of Lowicryl-ethanol (1:1) for 48 h, Lowicryl-ethanol (2:1) for 24 h, and fresh Lowicryl overnight. The samples were then placed in fresh Lowicryl, polymerized by indirect UV irradiation (360 nm) for 24 h at -18°C, and further polymerized at room temperature for 2 days. Ultrathin sections were collected on nickel grids with carbon-coated Formvar film. For immunolabeling, sections were incubated at room temperature on drops of the following reagents: PBS-0.1% BSA for 10 min; human fibrinogen fragment D (0.5 mg/ml) in PBS for 1 h; rabbit anti-human fibrinogen immune serum at a 1:100 dilution in PBS-0.1% BSA; and protein A-gold diluted 1:100 in PBS. Three washes (5 min each) were done in PBS-0.1% BSA between each step, and two (10 min each) were done in distilled water after the protein A-gold treatment.

Thin sections were contrasted with uranyl acetate. The specificity of the immunostaining procedures was established with the following controls: (i) fibronectin instead of fibrinogen and (ii) rabbit anti-fibronectin immune serum instead of rabbit anti-fibrinogen immune serum. Sections were examined on a 100 CX JEOL microscope.

RESULTS

Indirect immunofluorescence assay. Visualization of binding was performed with purified human fibrinogen free of cellular attachment factors, such as fibronectin, and with its plasmic degradation products, fragments D and E. After incubation with fibrinogen or fragment D, most of the conidia exhibited an intense labeling at their surface (Fig. 1A). However, the fluorescence was not evenly distributed: some cells showed a patchy distribution of labeling, whereas other cells showed faint fluorescence. When the immunofluorescence assay was performed with germ tubes, only the mother cell surface was labeled, no staining being detected at the hyphal surface (Fig. 1B and C). After incubation with fragment E, no labeling could be visualized either on mother cells or on germ tubes. Control experiments performed with rabbit anti-fibronectin immune serum instead of the primary antibody yielded negative results.

Binding assay with 125I-radiolabeled proteins. Since immunofluorescence showed that the binding of purified fibrinogen and fragment D was particularly associated with conidia, binding assays were performed with resting cells to confirm the binding site on the fibrinogen molecule. 125I-labeled fibrinogen and fragment D interacted with cells, whereas no reaction could be detected with radiolabeled fragment E under the same conditions. Incubation of conidia (1.5 x 10⁶) with the same amounts of radiolabeled proteins (5 x 10⁻¹² mol) resulted in the fixation of similar quantities of fibrinogen and fragment D, (7.44 ± 0.31) x 10⁻¹⁵ mol and (5.79 ± 0.22) x 10⁻¹⁵ mol, respectively, while only (0.15 ± 0.02) x 10⁻¹³ mol of fragment E interacted with the cells. After incubation with radiolabeled ligands, the composition of the material bound to conidia was also analyzed. The radiolabeled proteins bound to conidia were eluted from the fungal pellets by being heated in the presence of nonreducing electrophoresis sample buffer and then identified by polyacrylamide gel electrophoresis and autoradiography of the dried gels. An autoradiographic display demonstrated that the migration of 125I-polypeptide chains was identical to the migration of fibrinogen or its plasmic degradation products (data not shown).

Quantitative study of binding. The binding of fragment D to conidia increased over time (Fig. 2). Binding was half maximal after 30 min of incubation and reached a plateau at 90 min. Prolonging the incubation up to 120 min did not affect the amount of bound fragment D. Under the same conditions, fragment E reacted weakly with conidia, whatever the incubation time. In subsequent experiments, incubation of the cells with fragment D was routinely carried out for 90 min to allow maximal binding. To assess the satura-bility of fragment D binding to A. fumigatus conidia, we conducted a dose-response experiment with 0 to 6 μg of 125I-radiolabeled fragment D. The specificity of the binding
was studied in direct competition experiments in which 

\[ 125I \text{-fragment D} \] was added to the cells together with a large amount (100-fold molar excess) of unlabeled ligand. Specific binding increased as a function of fragment D concentration in the incubation medium, attesting to the saturability of the binding (Fig. 3A). A Scatchard plot analysis of the obtained data yielded a straight line, indicating the presence of only one class of receptor sites (Fig. 3B). The intersection of the regression line with the \( x \)-axis yielded the number of fragment D molecules bound per conidium. Assuming that all binding sites were occupied at saturation and that all fragment D molecules bound to the cell surface via one combining site, the mean number of receptors per cell was estimated to be approximately 1,200. Furthermore, a dissociation constant \( (K_d) \) of \( 2.2 \times 10^{-9} \text{ M} \) was calculated from the slope of the line.

**Sensitivity of the binding sites.** To characterize the biochemical nature of the binding sites, we heated cells at various temperatures or pretreated them with proteolytic enzymes and analyzed their ability to bind \( 125I \text{-fragment D} \) (Table 1). Heating the conidia at 56°C for 15 to 60 min did not influence the binding of fragment D. Conversely, when cells were heated at 80°C for 5 min, the binding capacity decreased dramatically. It was abolished after 15 min of heating at 80°C or 5 min of heating at 95°C.

Enzyme pretreatment of the conidia also resulted in the degradation of the fibrinogen-binding components. Pretreatment with a 100-μg/ml trypsin or chymotrypsin solution inhibited the specific binding of \( 125I \text{-fragment D} \) to \( A. \text{fumigatus} \) by 91.4% ± 2.8% or 94.3% ± 2.1%, respectively. Higher concentrations of enzyme solutions led to a complete loss of binding.

**Electron microscopy.** Electron microscopy performed on conidia at successive stages of development during the germination process revealed fibrinogen binding sites over the electron-dense outer layer of the conidial wall. However, since spore swelling was associated with stretching and disintegration of this layer, differences in the distribution of the fibrinogen receptors were observed. In resting conidia, gold particles were evenly distributed over this layer (Fig. 4A). In the early swelling stages, when the outer layer was disrupted, the labeling resided on electron-dense fragments, whereas exposed areas of the inner layer were not labeled or were poorly labeled (Fig. 4A and B). When these fragments were detached, they exhibited labeling on both sides (Fig. 4C). In more advanced stages of swelling, cell wall labeling was very scattered (Fig. 4A). The germ tube wall, which originated from the inner layer of the conidial wall, was slightly labeled (Fig. 4D).

Lowicryl-embedded \( A. \text{fumigatus} \) exhibited cell surface morphology comparable to that observed after embedding in Epon. Immunogold labeling revealed fibrinogen receptors mostly in the cell wall. Ultrathin sections of resting conidia revealed dense expression of these receptors in the inner part of the electron-dense layer (Fig. 5A). In contrast, fibrinogen receptors appeared to be distributed in all the cell wall layers of swollen conidia (Fig. 5B). More precisely, most of the gold particles were located on the inner face of the electron-dense layer and over the plasma membrane (Fig. 5C). However, the labeling was also detected in the cytoplasm, associated almost exclusively with the vacuolar...
TABLE 1. Influence of heat and enzyme pretreatment of conidia on fragment D binding

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Binding relative to the control (%)f</th>
</tr>
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<tbody>
<tr>
<td>None</td>
<td>100</td>
</tr>
<tr>
<td>56°C</td>
<td></td>
</tr>
<tr>
<td>15 min</td>
<td>100 ± 4.5</td>
</tr>
<tr>
<td>30 min</td>
<td>100 ± 4.5</td>
</tr>
<tr>
<td>60 min</td>
<td>100 ± 6.2</td>
</tr>
<tr>
<td>80°C</td>
<td></td>
</tr>
<tr>
<td>5 min</td>
<td>35 ± 3.4</td>
</tr>
<tr>
<td>15 min</td>
<td>0</td>
</tr>
<tr>
<td>30 min</td>
<td>0</td>
</tr>
<tr>
<td>95°C, 5 min</td>
<td>0</td>
</tr>
<tr>
<td>Trypsin</td>
<td></td>
</tr>
<tr>
<td>100 µg/ml</td>
<td>8.6 ± 2.8</td>
</tr>
<tr>
<td>500 µg/ml</td>
<td>0</td>
</tr>
<tr>
<td>1,000 µg/ml</td>
<td>0</td>
</tr>
<tr>
<td>Chymotrypsin</td>
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</tr>
<tr>
<td>100 µg/ml</td>
<td>5.7 ± 2.1</td>
</tr>
<tr>
<td>500 µg/ml</td>
<td>0</td>
</tr>
<tr>
<td>1,000 µg/ml</td>
<td>0</td>
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</tbody>
</table>

* Conidia were heated at different temperatures for 5 to 60 min and then cooled on ice. Other aliquots of the conidial suspensions were treated with the proteolytic enzyme trypsin or chymotrypsin for 30 min at 37°C, and the reaction was stopped by the addition of 1 mM PMSF, a serine protease inhibitor. The cells were then incubated with [125I]-fragment D. After 90 min of incubation, cell-associated radioactivity was measured as described in Materials and Methods. Results represent the mean ± standard error of triplicate determinations. The control was assayed in the absence of pretreatment.

A. fumigatus is a saprophytic fungus which binds to pathogenic aspergilli (7) and can mediate attachment to bronchoalveolar epithelial cells. Since the binding of fibrinogen to A. fumigatus appeared to be associated mainly with conidia, which are the infecting forms of the fungus, it seemed interesting to determine the in vitro conditions of the interaction.

Fibronectin is frequently present in commercially available preparations of fibrinogen. This multifunctional glycoprotein possesses numerous binding sites by which it links to other proteins, such as fibrinogen (19), or interacts with various pathogenic microorganisms (17). Thus, its possible role should be eliminated. Our finding of an unchanged binding pattern after further purification of fibrinogen by affinity chromatography demonstrated that fibrinogen is not involved in this interaction. Furthermore, as described for S. pyogenes (43) and C. albicans (1), the binding region seemed to be localized in the D domains of the fibrinogen molecule (Table 2). Labeling was detected by immunofluorescence at the surface of the fungal elements after incubation with fragment D, while no reaction could be observed with fragment E. Binding assays performed with radiolabeled fragments D and E confirmed that the D domains are involved in the binding of fibrinogen to A. fumigatus. This result is consistent with the spatial configuration of the fibrinogen molecule. Indeed, in the native protein, only the D domains are accessible. This result highlights the role of this interaction in the pathogenesis of the fungus.

Binding sites at the conidial surface seemed to possess characteristics of receptors. Binding was saturable and specific. A Scatchard plot analysis of equilibrium binding experiments revealed about 1,200 sites per cell, with a $K_d$ of $2.2 \times 10^{-7}$ M. This interaction is controlled by specific receptors with $K_d$ values similar to those reported for most of the microorganisms able to bind fibrinogen (Table 2). Conversely, low-affinity receptors for fragment D have been described on the surface of some mammalian cells, i.e., human platelets (3, 32, 34) and hemopoietic cells (29, 30) (Table 2). Endothelial cells (10) and fibroblasts (11) also interact with fibrinogen via low-affinity specific receptors, but in these cases binding sites are localized in the E domain.

As suggested by immunofluorescence and electron microscopy, the fibrinogen receptors seemed to be localized exclusively on conidia and on mother cells of germ tubes. Indeed, no reaction could be detected at the hyphal surface, in contrast to the binding pattern reported for C. albicans (1). Another striking difference between these two fungi is in their pathogenic mechanisms. The ability of C. albicans to undergo dimorphic transition from blastospore to germ tube is already known to be an important virulence factor in its conversion from a commensal organism to a pathogenic one. Moreover, this conversion correlates with an increased adherence to inert surfaces or to host cells and with the expression of fibrinogen receptors (40). In contrast, A. fumigatus is usually a saprophytic fungus which infects the host in its conidial form. Conidia must adhere to epithelial cells; otherwise, they are eliminated by mucus and the movements of the ciliary cells.

Finally, we attempted to characterize the receptors at the conidial surface. The thermolability of the binding sites as well as their sensitivity to proteolytic enzymes suggested their protein nature. However, we did not succeed in identifying the proteins by affinity chromatography with immobilized fibrinogen and ligand blotting with A. fumigatus soluble extracts. The ultrastructural localization of the receptors was analyzed by transmission electron microscopy for successive stages of development from resting conidia to germ tubes. In conidia, labeling appeared to be associated with the outer cell wall layer and progressively decreased during the germination process. This result could have been related to the previously described shedding of the outer layer of the conidial wall as the resting conidia mature (9). A more precise localization of the fragment D binding sites was achieved by labeling of thin sections of A. fumigatus conidia. Binding sites were detected particularly on plasma mem-
brane surfaces and membrane vacuoles. The isolation of A. fumigatus membranes and the partial characterization of their components (33) may provide the opportunity to study the membranous receptors and to compare these components with those extracted from cell wall fractions.

In conclusion, A. fumigatus presents on its conidia receptors for fibrinogen which could play an important role in the pathogenesis of aspergillosis. These receptors might mediate the attachment of conidia to damaged epithelia via fibrinogen deposited in response to the inflammatory reaction. This interaction might also protect conidia from phagocytosis by masking antigenic determinants and by inhibiting the deposition of C3 degradation products. Work is in progress to elucidate the implications of this interaction.
FIG. 5. Immunoelectron microscopic detection of fibrinogen fragment D binding sites on sections of resting (A) or swollen (B, C, and D) conidia processed after embedding in Lowicryl K4M. (A) In resting conidia, the labeling was mainly detected on the inner face of the electron-dense outer layer of the cell wall (×40,000). (B) The cell wall of swollen conidia was intensely labeled. Note the high density of receptors in the inner cell wall (×20,000). gl, glycogen; m, mitochondria; n, nucleus; v, vacuole. (C and D) High magnifications of swollen conidia (×40,000). Gold particles were distributed mostly along the plasma membrane and on the inner side of the electron-dense outer layer; however, vacuoles (v) and associated vesicles also showed labeling of their contents and limiting membranes (small arrows). In panel C, a close association between a vesicle and the inner part of the cell wall is clearly visible (large arrow). (E) Control experiment with nonspecific immune serum showing no gold particles (×15,000).
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REFERENCES


TABLE 2. Binding of fibrinogen to various prokaryotic or eukaryotic cells

<table>
<thead>
<tr>
<th>Cells studied</th>
<th>Binding site on the fibrinogen molecule</th>
<th>No. of receptors/cell</th>
<th>K_d (10^-8 M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus (16)</td>
<td>Fragment D1 (100 kDa)</td>
<td>2,130</td>
<td>0.99</td>
</tr>
<tr>
<td>S. aureus (39)</td>
<td>γ C-terminal sequence (15 residues)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>B. gingivalis (26, 27)</td>
<td>Plasmin-sensitive site of the coiled-coil region between the D and E domains</td>
<td>500–1,500</td>
<td>2.7–9.8</td>
</tr>
<tr>
<td>B. intermedius (26, 28)</td>
<td>Fragment D1 (100 kDa)</td>
<td>3,500</td>
<td>3.4</td>
</tr>
<tr>
<td>S. pyogenes (42, 43)</td>
<td>Fragment D (85 kDa)</td>
<td>ND</td>
<td>0.1–0.5</td>
</tr>
<tr>
<td>C. albicans (1)</td>
<td>Fragment D (85 kDa)</td>
<td>6,000</td>
<td>5.2</td>
</tr>
<tr>
<td>Human platelets (32)</td>
<td>Fragment D (85 kDa)</td>
<td>1,200</td>
<td>0.22</td>
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<tr>
<td>Human platelets (3)</td>
<td>Fragment D (85 kDa)</td>
<td>ND</td>
<td>13</td>
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<td>Human platelets (34)</td>
<td>Fragment D (85 kDa)</td>
<td>ND</td>
<td>8–17</td>
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<td>Human platelets (30)</td>
<td>Fragment D (85 kDa)</td>
<td>30,000–50,000</td>
<td>50</td>
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<td>Raji cells (29, 30)</td>
<td>Fragment D (85 kDa)</td>
<td>149,000</td>
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<td>Fibroblasts (11)</td>
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* ND, not determined.