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Electron Microscopic Localization of Receptors for Aggregated β_2-Microglobulin on the Surface of Beta-Hemolytic Streptococci

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The presence and location of receptors for aggregated human β_2-microglobulin (β_2m) on the surface of group A, C, and G streptococci were studied by electron microscopic techniques. Ferritin-conjugated aggregates of human β_2m were used in direct binding experiments. Ferritin-conjugated antibodies against β_2m were employed in a two-step indirect binding assay where the streptococci were incubated with unlabeled β_2m aggregates before the addition of antibodies. Similar results were obtained with these two methods. Among tested group C and G strains, some showed binding of β_2m, whereas others were negative. In group A streptococci, β_2m binding was localized to filamentous structures typical of M protein. In two M protein-negative group A strains, the reactivity was heterogeneous, revealing a majority of unlabeled, but also some heavily labeled streptococci. Morphologically, these β_2m-binding bacteria exhibited M protein-like projections in contrast to the smooth surfaces of unlabeled cells.

The discovery of the reaction of protein A from *Staphylococcus aureus* with the Fc region of immunoglobulin G (IgG; 6) stimulated an extensive search for structures with similar binding specificities in other gram-positive cocci. A series of surface structures with receptor specificities for different mammalian plasma proteins have been detected in beta-hemolytic streptococci. These proteins include IgG (8, 12), IgA (5, 17), albumin (11, 13, 23), haptoglobin (7), fibrinogen (10, 19), and aggregated β_2-microglobulin (β_2m) (3, 4, 9).

Using ferritin-conjugated IgG from different species, some workers have studied the ultrastructural location of the IgG Fc receptor in strains of group A streptococci (14, 15). The aim of the present paper was to demonstrate by similar methods the ultrastructural arrangement of the receptors for aggregated β_2m in strains of beta-hemolytic streptococci of Lancefield groups A, C, and G.

MATERIALS AND METHODS

Bacterial strains. The following streptococcal strains were used in the studies. Group A streptococci included strain T1 (M^+) and its M protein-negative variant T1 (M^-) (3) and strain T12 (M^+) and its M protein-negative variant T12 (M^-) (3). Strains AW16, AW25, and AW43 (M type 60) were kindly supplied by L. W. Wannamaker, Minneapolis, Minn. Group C streptococcal strains C14, C25, and C42 and group G streptococcal strains G43 and G148 (8) were obtained from routine cultures at the Clinical Microbiology Laboratory, University Hospital, Lund, Sweden. The strains were stored at −80°C in Todd-Hewitt broth supplemented with 50% fetal calf serum and subcultured on blood agar consisting of blood agar base (Oxoid Ltd., London, England) supplemented with 4% defibrinated horse blood. For binding studies, the bacteria were grown in Todd-Hewitt broth at 37°C overnight, centrifuged, and washed twice with phosphate-buffered saline (pH 7.2) containing 0.2% sodium azide.

β_2m. Human β_2m was purified from urine (1), and aggregates were prepared as described previously (3).

Conjugation of proteins with ferritin. Aggregated human β_2m was conjugated with ferritin (crystallized five times; Research Institute for Vaccines, Dessau, German Democratic Republic) by the glutaraldehyde method (22). The IgG fraction from a goat antihuman β_2m, prepared by precipitation of the globulin with ammonium sulfate and chromatography on DEAE-cellulose, as well as the $F(ab')_2$ fragment of this antibody (2), was conjugated with ferritin in the same way.

The conjugation of proteins with ferritin was checked by agar electrophoresis followed by staining of the slides with amido black 10 B and for the Prussian blue reaction. The conjugates were purified from the reactants by chromatography on a column of Bio-Gel A-1.5m (Bio-Rad Laboratories, Richmond, Calif.), using phosphate-buffered saline for elution (21). The purified fractions were pooled, concentrated against Aquacide (Calbiochem, La Jolla, Calif.), and sterilized by filtration (0.45-μm pore size; Millipore Corp., Bedford, Mass.).

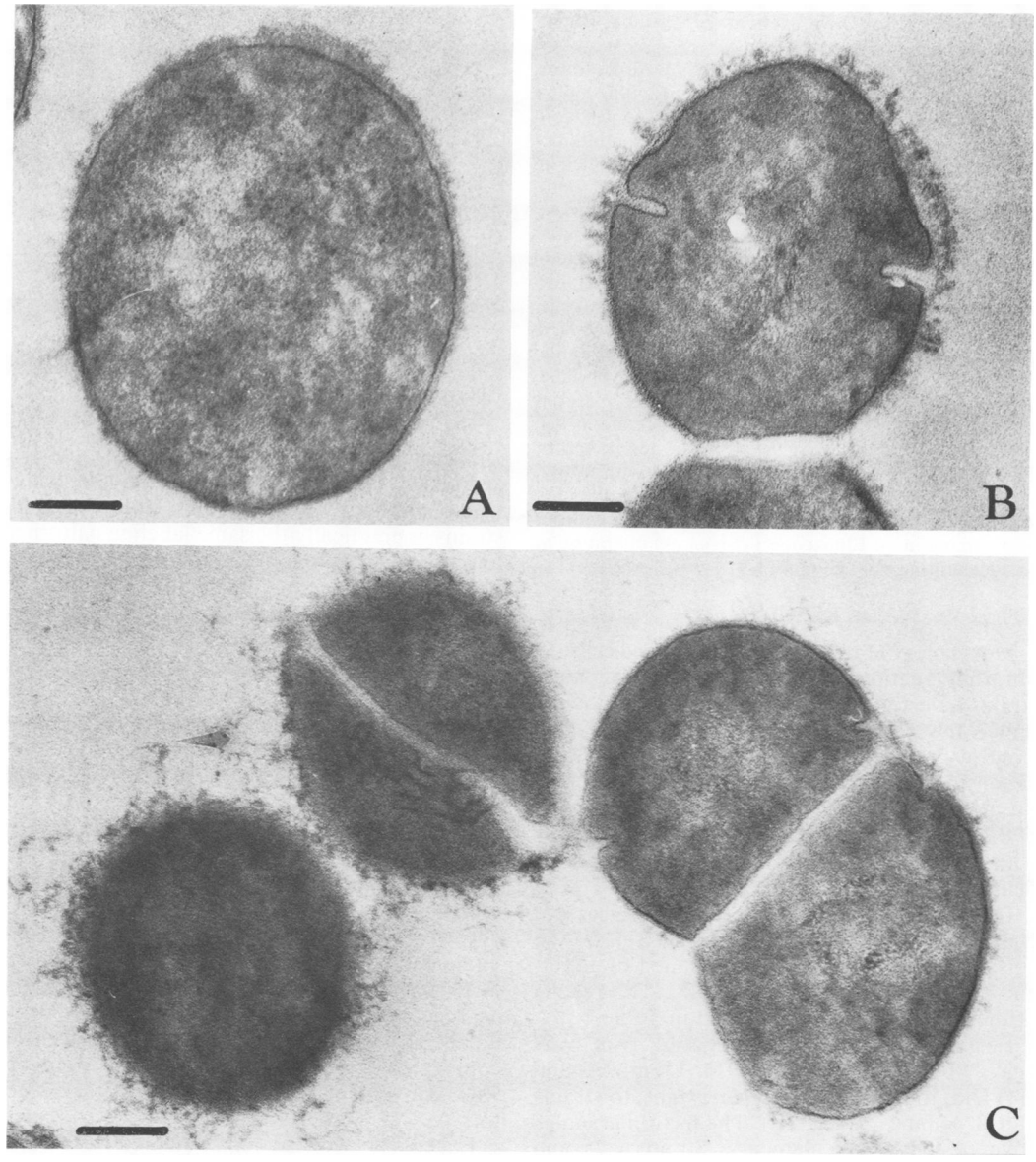

FIG. 1. Morphology of the surface of untreated beta-hemolytic streptococci. (A) Strain G148 with a nearly smooth surface, (B) strain T12 (M^+) with long filaments, (C) strain T12 (M^-) with both smooth and filament-covered cells. Bar, 0.2 μm.

Demonstration of receptors for aggregated β_2m. For the demonstration of receptors for aggregated β_2m, two methods were used: (i) the binding of ferritin-conjugated β_2m and (ii) the binding of unlabeled β_2m followed by the interaction of the bacteria with ferritin-conjugated whole antibody or F(ab')$_2$ fragment of anti-β_2m. Washed sediments (about 0.1 ml) of the bacteria were incubated with 0.2 ml of these reagents and incubated for 1 h each at 37°C under slight agitation.

Controls. As negative controls, cells were fixed for electron microscopy without incubation with conjugates, or they were incubated with a 1% (wt/vol) ferritin solution under otherwise identical conditions.

Electron microscopy. Incubated cells were washed in phosphate-buffered saline and 0.2 M cacodylate buffer, embedded in 2% Noble agar (Difco Laboratories, Detroit, Mich.), and fixed for 30 min at room temperature in 2.5% (vol/vol) glutaraldehyde in 0.075 M cacodylate buffer (with 0.17 M sucrose + 0.05% $CaCl_2$), pH 7.0. After two washings, each in 0.2 M cacodylate buffer and RK buffer (16) for 10 min, the embedded material was fixed with 1% (wt/vol) OsO_4 in RK buffer containing 1% (wt/vol) tryptone at 4°C overnight. After repeated washings in RK buffer, the material was contrasted with 1% (wt/vol) uranyl acetate in RK buffer in the dark for 1 h at room temperature, dehydrated in graded acetone contrasted with uranyl acetate and phosphotungstic acid (24), and embedded in Epon. Ultrathin sections were examined without poststaining in an Elmiscope I (Siemens) operating at an accelerating voltage of 80 kV.

RESULTS

Morphology of the surface of untreated cells. In ultrathin sections, the cell surface of most strains investigated was nearly smooth, showing only a few scattered fuzzy filaments (Fig. 1A). Contrary to this, cells of strain T12 (M^+) were heavily coated with long filaments (Fig. 1B). Strain T12 (M^-), an M protein-negative variant of the former strain, contained a majority of cells with a smooth surface and a few cells with long filaments (Fig. 1C).

Demonstration of receptors for aggregated β_2m. The presence of receptors for β_2m on the surface of streptococci was examined by different electron microscopic methods. The results of these studies are summarized in Table 1.

Ferritin-conjugated aggregated β_2m was heavily bound to strains T12 (M^+) (Fig. 2A) and G43 and, to a somewhat lesser extent, to strains T1 (M^+) and C42 (Fig. 2B). The ferritin granules covered the whole filaments. Strains C25 and G148 (Fig. 2C) exhibited only a very weak or no labeling. Cells of strains T1 (M^-) and T12 (M^-) showed no uniform labeling pattern. Most of these cells were unlabeled, although some cells (2 to 5%) exhibited a moderate or heavy labeling in the same arrangement as seen in strains T1 (M^+) and T12 (M^+) (Fig. 2D).

TABLE 1. Binding of ferritin-conjugated or unconjugated aggregated β_2m to groups A, C, and G streptococci

Streptococcal group and strain	Fe-β_2m[a]	Binding of unconjugated β_2m	
		Fe-anti-β_2m (IgG antibody)	Fe-anti-β_2m [F(ab')$_2$ fragment]
A			
T1 (M^+)	++	+++	ND[b]
T1 (M^-)	−/++[c]	−/++	ND
T12 (M^+)	+++	++	ND
T12 (M^-)	−/+++	−/++	ND
C			
25	−	++	−
42	++	++	+
G			
43	+++	++	++
148	−	++	−

[a] Fe, Ferritin.
[b] ND, Not done.
[c] −/++, The majority of cells are unlabeled, a few cells are labeled.

The incubation of the bacteria with unlabeled aggregated β_2m followed by ferritin-conjugated whole antibody of anti-β_2m resulted in most strains in practically the same labeling pattern as found with ferritin-conjugated β_2m (Fig. 3A and B and 4A and B). Strains C25 and G148, however, exhibited, in this experiment, a strong labeling (Fig. 4D and E). No ferritin binding was observed, however, for these strains when ferritin-conjugated F(ab')$_2$ fragments of anti-β_2m were used in the second incubation step instead of whole antibodies (Fig. 4F). For strains C42 and G43, all three procedures gave identical results (Fig. 4A to C).

DISCUSSION

In this paper, the presence and location of receptors for β_2m on the cell surface of streptococci of Lancefield groups A, C, and G were demonstrated by electron microscopic methods. Both the direct binding of ferritin-conjugated aggregated β_2m and a two-step technique using unlabeled aggregated β_2m followed by ferritin-conjugated anti-β_2m IgG or its F(ab')$_2$ fragment were convenient for the detection of the receptor.

Except for two reactions obtained with whole anti-β_2m antibodies, identical results were obtained. The two false-positive reactions of strains C25 and G148 were caused by binding of the IgG antibody to the Fc receptor. Using F(ab')$_2$ fragments of the same antibody, we found that the corresponding reactions were negative. These findings again demonstrate that

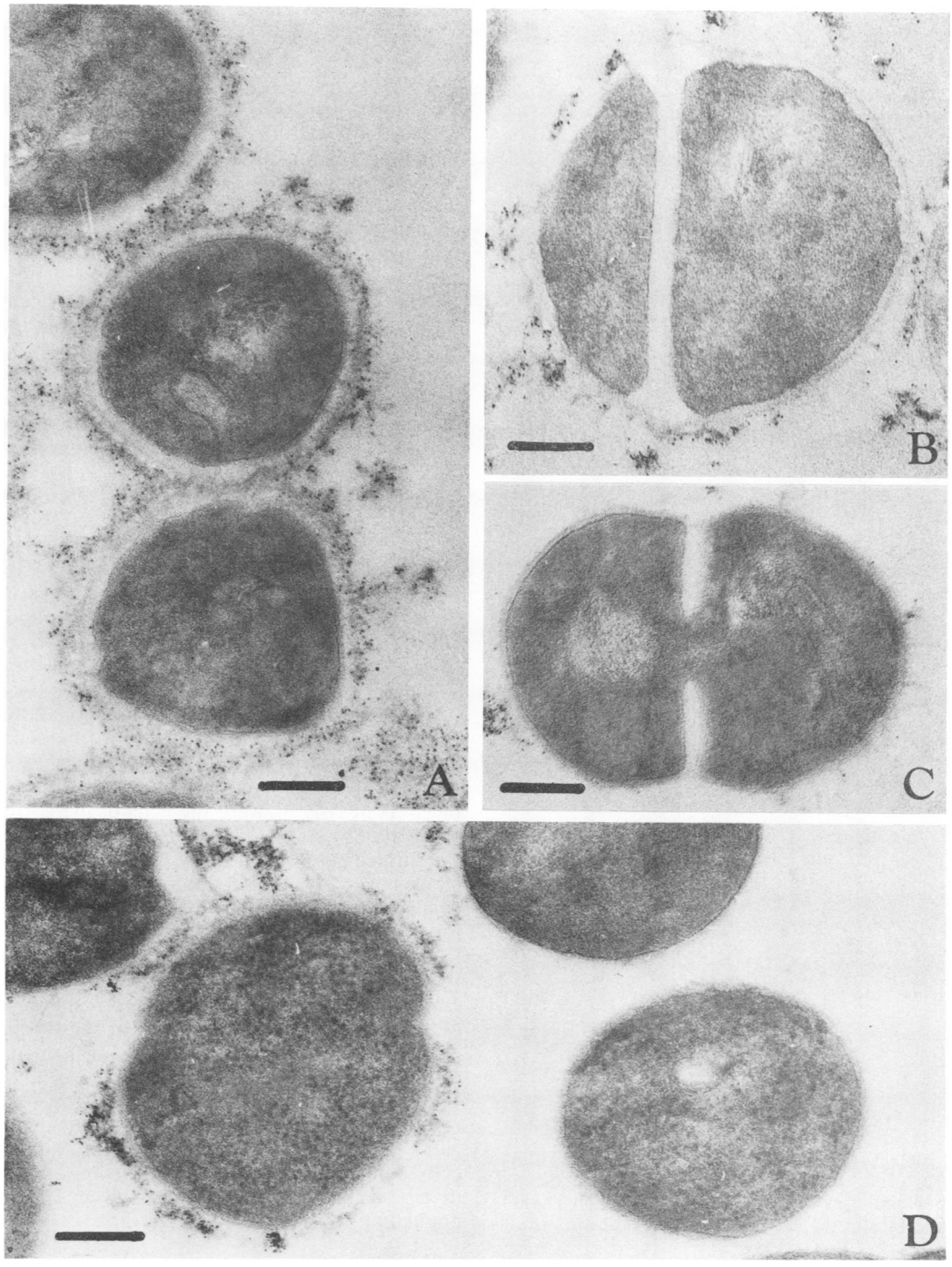

FIG. 2. Binding of ferritin-conjugated aggregated β_2m to streptococci. (A) Strain T12 (M^+); (B) strain C42; (C) strain G148, no binding; (D) strain T12 (M^-), binding only on a few cells. Bar, 0.2 μm.

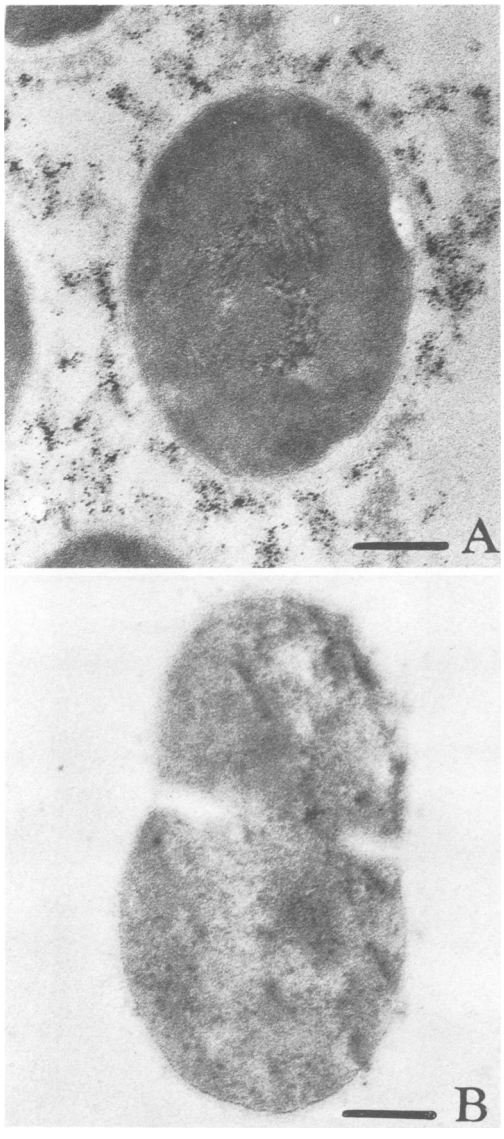

FIG. 3. Binding of unconjugated aggregated β_2m to streptococci followed by reaction with ferritin-conjugated anti-β_2m (whole antibody). (A) Strain T1 (M^+); (B) strain T1 (M^-), no labeling. Bar, 0.2 μm.

the binding of aggregated β_2m does not correlate with IgG binding (9).

Our results are in agreement with those of previous investigations using radiolabeled β_2m for the detection of this receptor (3, 4, 9). The advantage of electron microscopy is the direct visualization of the receptor sites on the cell surface, revealing both the exact ultrastructural locations and the properties of individual cells within a population. It could be shown that the receptor is located on the filamentous protrusions of the cell surface. The arrangement of the ferritin granules strongly resembles that found in studies on the location of the M protein of group A streptococci (18, 20). Results obtained in binding experiments with M protein-positive (M^+) and their M protein-negative (M^-) variants suggest that aggregated β_2m is bound to M protein (3). The present work further supports the notion that the β_2m-binding structures are located on the same filamentous structures as the M protein. The reactivity of some group C and G strains suggests that the cell surface of these strains contains proteins with a structure similar to that of the M protein.

It could also be shown that the reaction of the cells in the M^- variant strains is not homogenous. The majority of cells remained unlabeled, but a small proportion of cells was ferritin labeled as heavily as were the cells of the corresponding M^+ strain. This is in accordance with the surface morphology of the M^- strains, which show both cells with a smooth surface and a small number of cells with long filamentous protrusions. In binding experiments with radiolabeled aggregated β_2m, the M^- strains used in the present study showed an uptake of 5 to 15% compared with about 70% for the corresponding M^+ strains (3). These figures appear reasonable in comparison with the proportion of filament-carrying and ferritin-labeled M^- bacteria (2 to 5%). The present results suggest that the low reactivity of M^- strains with radiolabeled aggregated β_2m is also caused by binding to the few cells containing filamentous protrusions rather than by uniformly weak binding to all cells of the population.

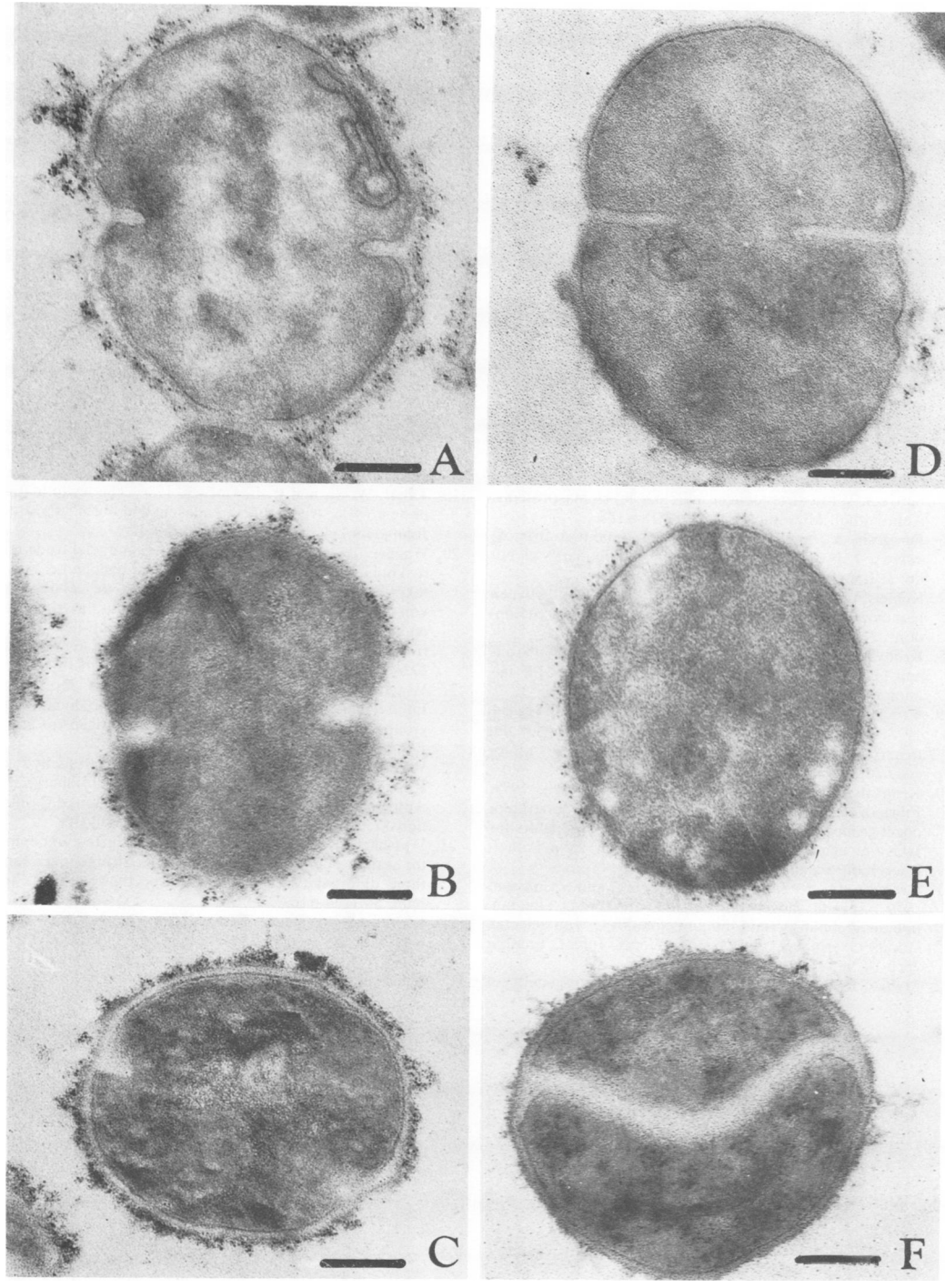

FIG. 4. Comparative study of the binding of aggregated β_2m to strain G43 (A to C) and strain C25 (D to F) by different labeling procedures. (A and D) Ferritin-conjugated β_2m, (B and E) β_2m followed by ferritin-conjugated anti-β_2m (whole antibody), (C and F) β_2m followed by ferritin-conjugated anti-β_2m F(ab')$_2$ fragment. The labeling in (E) is caused by the reaction of the antibody with the Fc receptor. Bar, 0.2 μm.

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