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Binding of Aggregated Human β_2-Microglobulin to Surface Protein Structure in Group A, C, and G Streptococci

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A novel mammalian-microbial "short circuit" has been demonstrated between aggregated human β_2-microglobulin and group A, C, and G streptococci. Bacteria belonging to nine gram-positive and three gram-negative species were tested for binding of radiolabeled β_2-microglobulin. All 10 individual strains of group A streptococci showed a high degree of reactivity with aggregated human β_2-microglobulin. Among 27 group C and 28 group G streptococci, 9 and 6 strains, respectively, were highly reactive, whereas the remaining strains showed a lower, but definite level of β_2-microglobulin binding. Of 11 group B streptococci, 4 were slightly positive. All strains among the other eight species were completely negative. Simultaneous testing of A, C, and G streptococci for immunoglobulin binding showed a lack of correlation between type II and III Fc reactivity and β_2-microglobulin binding. There was no inhibition of uptake of aggregated β_2-microglobulin to reactive strains when excess amounts of human immunoglobulin were added. The β_2-microglobulin-binding surface structure was found to be markedly sensitive to trypsin digestion. The relative trypsin resistance of the immunoglobulin-binding protein in the digestion experiments further demonstrated the dissociation between these two reactivities.

β_2-microglobulin is a small protein originally isolated from human urine (4). It is similar to immunoglobulin domains with respect to size and amino acid sequence (16, 18), and it occurs linked to major histocompatibility antigens of various mammalian species (cf. reference 5). These observations and the fact that the larger subunits of such antigens (HLA and H2) seem to be arranged in domains (17, 19) suggest a structural relationship between immunoglobulins and major histocompatibility antigens. Similarities have also been shown in the expression of functional characteristics. Aggregated human β_2-microglobulin was capable of fixing complement, and, when coated onto sheep erythrocytes, human β_2-microglobulin promoted binding to guinea pig peritoneal macrophages (15). Cytophilic properties have been noted also for mouse and rat lymphocytes and polymorphonuclear leukocytes (1). Furthermore, aggregated heavy chains of H2 antigens, and to a lesser extent also monomeric chains, showed an affinity for staphylococcal protein A, competing with the immunoglobulin receptor (17).

Immunoglobulin Fc-binding properties of gram-positive cocci were first discovered in staphylococci (7) and later in streptococcus groups A, C, and G (10). Recent studies have indicated major differences in specificities of these immunoglobulin-binding bacteria (14). Three major types of Fc-reactive structures were defined: type I, represented by staphylococcal protein A; type II, represented by streptococcus group A; and type III, represented by both group C and group G streptococci (14). In view of the similarity between the CH2 domain, carrying structures with affinity for gram-positive cocci, and β_2-microglobulin, this latter component of the histocompatibility antigen might also show an affinity for Fc-binding gram-positive cocci. The present experiments, designed to investigate this possibility, revealed a marked reactivity between aggregated β_2-microglobulin and structures on group A, C, and G streptococci different from immunoglobulin-binding components.

MATERIALS AND METHODS

Bacterial strains. A total of 166 strains of human pathogenic bacteria was included in the study. All strains were obtained consecutively from clinical specimens sent to the Clinical Microbiology Laboratory, University Hospitals, Lund, Sweden, for bacteriological examination. The bacterial species and numbers of strains were as follows: *Staphylococcus aureus*, 16; *Staphylococcus epidermidis*, 11; *Staphylococcus saprophyticus*, 10; group A streptococci, 10; group B streptococci, 11; group C streptococci, 27; group G streptococci, 28; *Streptococcus faecalis*, 10; *Streptococcus*

pneumoniae, 12; *Escherichia coli*, 10; *Klebsiella pneumoniae*, 10; and *Pseudomonas aeruginosa*, 11. The strains were subcultured on blood agar plates and then grown in tryptone broth or Todd-Hewitt broth for studies of β_2-microglobulin binding.

β_2-Microglobulin. Human and rabbit β_2-microglobulins were purified from urine specimens as described previously (3, 4). The proteins were radiolabeled with ^{125}I by using the chloramine-T method (8).

Aggregation of β_2-microglobulin. Preparations of aggregated human β_2-microglobulin were obtained by using glutaric dialdehyde as a coupling reagent, by the method of Avrameas (2). Equal volumes of β_2-microglobulin (2 mg/ml) and 1.6% glutaric dialdehyde in 0.1 M phosphate buffer, pH 7.5, were mixed and incubated at room temperature for 1 h. One volume of 1% sodium metabisulfate was then added, and the reaction mixture was subjected to dialysis or gel filtration. No precipitate was formed during the aggregation.

Gel filtration. Aggregated β_2-microglobulin was chromatographed on a Sephadex G-200 column (diameter, 25 mm; height, 900 mm) equilibrated with phosphate-buffered saline (0.12 NaCl–0.03 M phosphate, pH 7.3–0.02% sodium azide) containing 0.1% Tween 20. Fractions containing 3.75 ml were eluted at a speed of 12 ml/h.

Radiolabeled human IgG. Human polyclonal immunoglobulin G (IgG) (lot 62842; A. B. Kabi, Stockholm, Sweden) was radiolabeled with the chloramine-T method (8, 13) and used for measuring the Fc-binding capacity of strains as described previously (14).

β_2-Microglobulin-binding assay. Overnight cultures of bacterial strains in tryptone or Todd-Hewitt broth were washed twice in phosphate-buffered saline and suspended to 10^9 bacteria per ml, as calculated from optical density at 620 nm values on diluted samples in a Beckman CP-1 calorimeter. The final suspension was made in phosphate-buffered saline with 0.1% human serum albumin (A. B. Kabi) and 0.05% Tween 20 added.

To 0.2-μg portions of β_2-microglobulin preparations 200 μl of bacterial suspension was added and left at room temperature for 1 h. A 2-ml quantity of phosphate-buffered saline containing albumin and Tween 20 was then added, the bacteria were centrifuged, and the supernatant was discarded. Radioactivity was measured in an LKB-Wallac 1270 Rackgamma gamma radiation counter (A. B. Biotec, Stockholm, Sweden). The uptake of radiolabeled β_2-microglobulin or human immunoglobulin was expressed as percent radioactivity remaining in the pellet.

Trypsin digestion. Increasing amounts of trypsin (catalog no. T-8253; Sigma Chemical Co., St. Louis, Mo.) were added to bacterial suspensions (10^9 bacteria per ml in 0.25 M phosphate buffer, pH 7.5), followed by incubation at 37°C for 20 min. Digestion of bacterial proteins was stopped by the addition of Trasylol (Bayer) and subsequent washings. Resuspended bacteria were then tested for uptake of β_2-microglobulin and human immunoglobulin. In some experiments, the amount of trypsin added was kept constant and the digestion time was varied between 5 and 120 min.

Ultrasonic treatment. Samples (1 ml) of bacterial suspensions were treated with ultrasound by using a Branson B-12 Sonifier with microtip attachment (Branson Power Co., Danbury, Conn.).

RESULTS

Monomeric β_2-microglobulin-binding studies. Radiolabeled human and rabbit β_2-microglobulin preparations were added to *S. aureus* strains and to group A, C, and G streptococci in binding assays. No uptake of these monomeric preparations was noted to any of the strains.

Binding of aggregated human β_2-microglobulin to bacteria. Glutaraldehyde-aggregated, radiolabeled human β_2-microglobulin with a relative molecular weight of 275,000 on gel filtration was added to suspensions of bacteria in binding assays. In all, 166 strains of 12 species were tested. Three species, group A, C, and G streptococci, showed marked binding of aggregated β_2-microglobulin (Fig. 1). All 10 group A streptococci were reactive, with binding of 70 to 100% of added β_2-microglobulin. The reactivity with group C and G streptococci was more heterogeneous, with an uptake ranging from 0 to 100% (Fig. 1). Eight bacterial species were completely negative, whereas 4 of 11 group B streptococcal strains showed slightly elevated levels.

Effect of aggregate size on binding. Aggregated human β_2-microglobulin was gel filtered on a calibrated Sephadex G-200 column. The major part of the protein was eluted as 275,000-molecular-weight complexes, with decreasing amounts of smaller aggregates down to monomers. Fractions with different aggregate size were collected, and the binding to two group A streptococci was tested (Fig. 2). There was a marked increase in binding of β_2-microglobulin

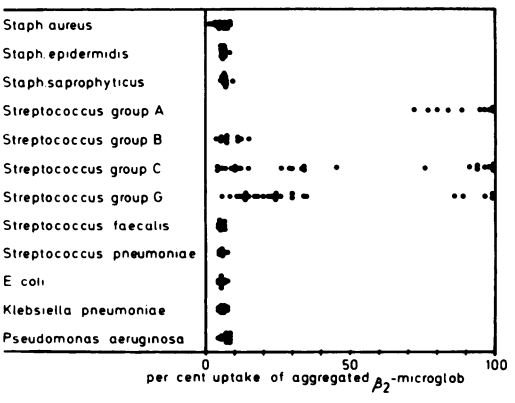

FIG. 1. *Quantitative binding of aggregated β_2-microglobulin (0.2-μg portions) to bacterial strains of 12 different species.*

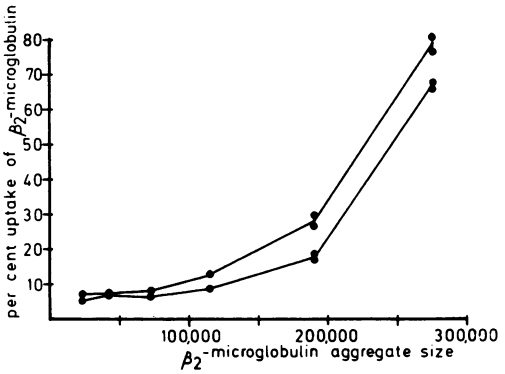

FIG. 2. *Quantitative binding of β_2-microglobulin of various aggregate sizes (0.2 μg in each test) to two strains of group A streptococci.*

with increasing molecular aggregation. Smaller aggregates below an apparent molecular weight of 100,000 showed only slight reactivity.

Effect of incubation time on binding. The influence of incubation time at room temperature on the binding of aggregated, radiolabeled β_2-microglobulin was studied by using two group A streptococcal strains (A1 and A207) (Fig. 3). One strain (A1) showed a level of uptake characteristic of most group A strains. The other strain (A207) was included as a representative of strains with a lower level of binding. The A1 strain with a high uptake tended to reach equilibrium at a faster rate, with almost maximal binding after only 10 min of incubation. The A207 strain with a lower uptake did not reach its equilibrium even after 120 min of incubation. The results indicate that different levels of binding also seem to reflect differences in speed or strength of association rather than quantitative binding differences only.

Correlation to immunoglobulin Fc reactivity. Group A, C, and G streptococci, known to carry type II and type III Fc-binding proteins (14), were tested simultaneously for uptake of aggregated β_2-microglobulin and normal human IgG (Fig. 4). There was a complete dissociation between the two binding properties, suggesting that different bacterial structures are involved. Three distinct groups of G streptococci were seen (Fig. 4C). One group was highly reactive with both ligands, another group was strongly IgG reactive, and a third group was IgG negative, the latter two with low binding of β_2-microglobulin.

Inhibition experiments. Most group A, C, and G streptococci have the capacity to bind normal human immunoglobulin (10, 14) and to bind human fibrinogen as well (9, 20; G. Kronvall, C. Schönbäck, and E. Myhre, manuscript in preparation). Inhibition experiments were performed to evaluate possible steric interactions between immunoglobulin and β_2-microglobulin binding. Amounts of normal human IgG, ranging from 0.01 to 100 μg, were mixed with aggregated, radiolabeled β_2-microglobulin (0.2 μg) before the addition of test bacteria in the binding assays. Two group A streptococci (A1 and A207), one group C strain (C36), and one group G strain (G22) were included, representing various types of IgG and fibrinogen reactivities. There was no influence on the uptake of β_2-microglobulin by immunoglobulin over the entire concentration range in these inhibition experiments.

Trypsin sensitivity of β_2-microglobulin-binding structures. Digestion experiments were performed to detect possible trypsin sensitivity of the binding structures and to compare these with the Fc-binding protein. With increasing amounts of trypsin (2.5 to 200 μg/ml of bacterial suspension) there was a rapid fall in reactivity of group A, C, and G streptococci with aggregated β_2-microglobulin (Fig. 5A). The loss of reactivity was almost complete when amounts as small as 20 μg were used. On the other hand, the Fc-binding properties were completely unchanged with these amounts of trypsin over the entire range (Fig. 5B).

Effect of ultrasonic treatment on binding structures. Suspensions of two group A streptococcal strains, one group C, and one group G

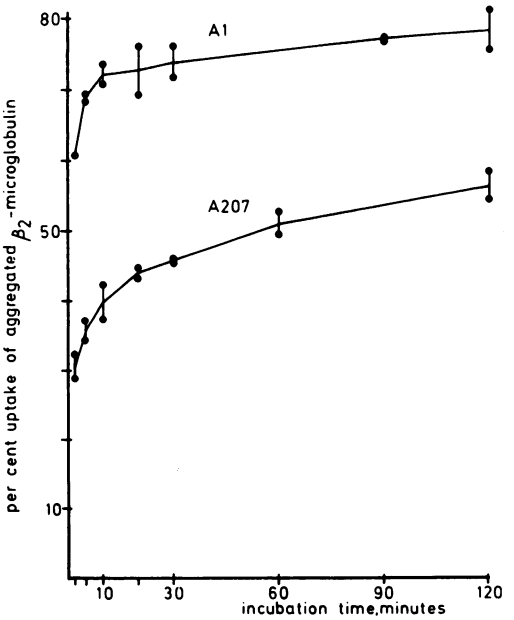

FIG. 3. *Effect of incubation time on the binding of aggregated β_2-microglobulin to group A streptococci (strains A1 and A207).*

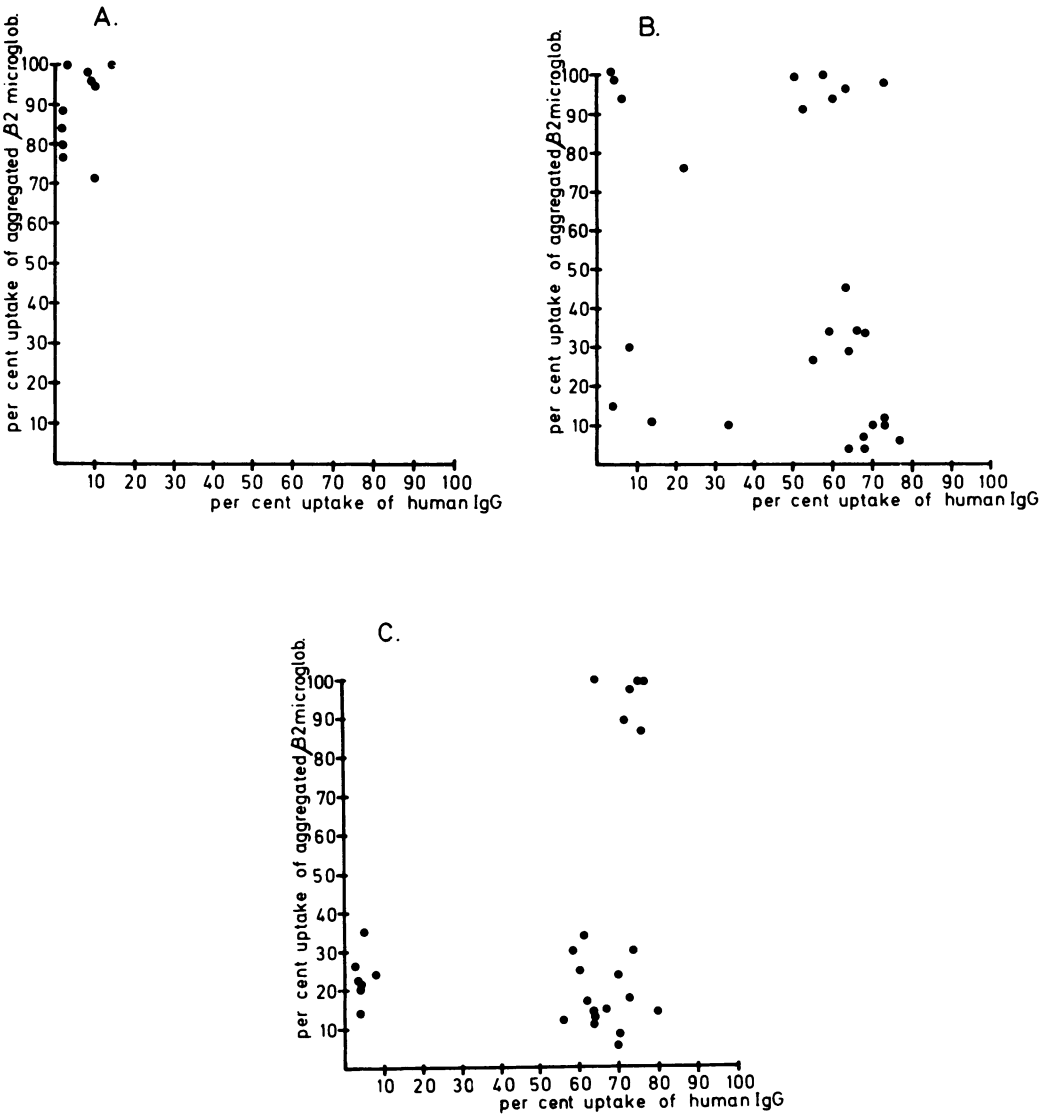

FIG. 4. *Correlation between uptake of normal human IgG and aggregated β_2-microglobulin in individual streptococcal strains of human origin. (A) Group A streptococcal strains; (B) group C streptococcal strains; (C) group G streptococcal strains.*

strain were treated with ultrasound for 5, 15, and 60 s. The suspensions were then washed, and the capacity to bind aggregated β_2-microglobulin was measured. The IgG-binding capacity was also determined for comparative purposes. Smears of the suspensions were stained with acridine orange (11), and the degree of aggregation was estimated microscopically. Both group A streptococci showed a loss of β_2-microglobulin binding after ultrasonic treatment (Fig. 6A). The effect was most marked on the A207 strain, whereas the binding to the A1 strain was not so drastically reduced (Fig. 6A). There was no measurable effect of ultrasonic treatment on the capacity of strain C36 or G22 to bind aggregated β_2-microglobulin. In control experiments where the uptake of normal human immunoglobulin was measured, there were no effects noted under the present conditions of ultrasonic treatment (Fig. 6B).

DISCUSSION

Structural and functional similarities between β_2-microglobulin and the constant domains of human IgG prompted the present investigation of possible interactions between β_2-microglobu-

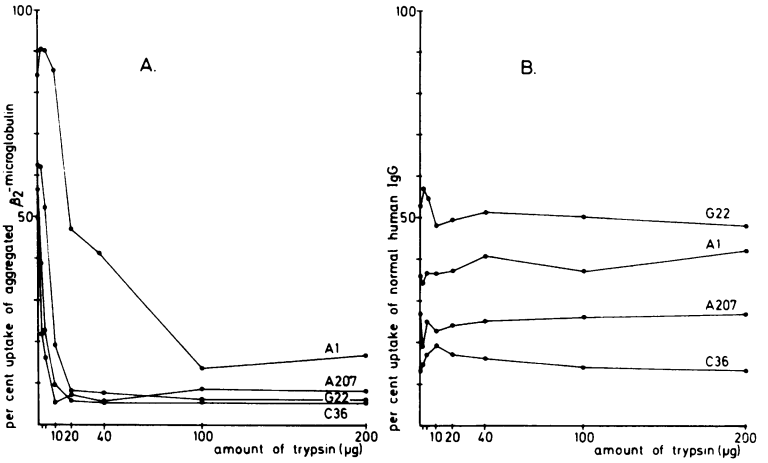

FIG. 5. *Effect of trypsin digestion of streptococcal strains on the capacity to bind aggregated human β_2-microglobulin (A) or normal human IgG (B).*

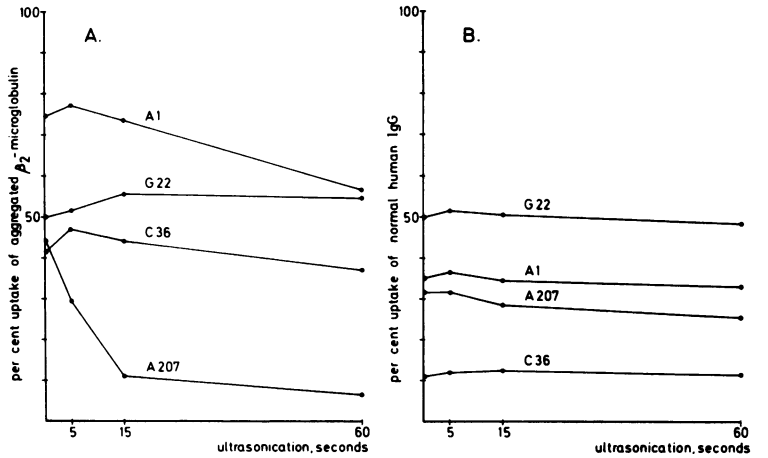

FIG. 6. *Effect of ultrasonic treatment on the binding of aggregated β_2-microglobulin (A) and normal human IgG (B).*

lin and Fc-reactive, gram-positive cocci. Bacteria with defined IgG-binding properties were included, as well as other bacterial species. Binding of monomeric as well as aggregated β_2-microglobulin was tested for 166 strains belonging to 12 different species (Fig. 1). Only group A, C, and G streptococci showed reactivity with β_2-microglobulin, and only aggregates of β_2-microglobulin with apparent molecular weights above 100,000 showed significant binding.

The most striking feature of the group A, C, and G streptococcal reactivity with aggregated β_2-microglobulin was the lack of correlation with Fc-binding structures type II and type III in the same bacterial species. This was shown in direct studies of binding with a complete lack of correlation (Fig. 4), in inhibition experiments with no effect of added IgG, and in differences in trypsin and ultrasound sensitivity of the bacterial surface proteins involved (Fig. 5 and 6). Therefore, the results of our studies do not suggest any direct similarity between β_2-microglobulin and IgG CH2 domain structures with binding affinity for gram-positive bacteria. Such a similarity has previously been proposed to exist between H-2 heavy chains and immunoglobulin on the basis of a similar protein A reactivity (17). We have discovered a surface marker on group A, C, and G streptococci different from immunoglobulin-binding structures.

When group A, C, and G streptococci were compared with each other concerning the characteristics of β_2-microglobulin binding, the group A streptococci appeared different from group C and G streptococci. Group A streptococci were all highly reactive (Fig. 1 and 4A). Ultrasonic

treatment caused a significant decrease in the reactivity of two group A strains, whereas one group C strain and one group G strain were unaffected. Group C and G streptococci segregated into one highly reactive group and one low- or nonreacting group. Immunoglobulin Fc-binding characteristics show a similar correlation with a type II reactivity defined in group A streptococci and a type III reactivity among both group C and group G streptococci. The nonidentity of β_2-microglobulin binding and IgG Fc binding has been demonstrated by other tests. The similar correlation to streptococcal groups might therefore only reflect a more recent evolutionary divergence of group C and G streptococci from the common origin of all three group A, C, and G species.

The new surface marker on group A, C, and G streptococci, detected by the binding of aggregated β_2-microglobulin, seems to be a protein, as judged by trypsin digestion experiments (Fig. 5). The marked trypsin sensitivity is similar to that of M-proteins and differs from that of other streptococcal surface proteins, such as the T-antigens and the R-antigens. A direct association with M-protein structures is therefore possible. This might explain the reported M-protein-associated adherence of streptococci to epithelial cells (6).

Unlike immunoglobulin Fc and fibrinogen binding, there is no binding of the monomeric form of the ligand to the β_2-microglobulin-reactive protein in the present test system. In vivo, β_2-microglobulin is probably located on all nucleated cells. Its function might therefore be associated with a nonsoluble state. The emergence of a receptor on streptococci might be due to an evolutionary pressure on a reactivity with cell membrane-bound, nonsoluble, functionally aggregated β_2-microglobulin. The possibility of false-negative results with monomeric β_2-microglobulin in the assay should also be considered. If the binding site on β_2-microglobulin is sensitive to iodination, only aggregates containing both intact and ^{125}I-labeled molecules would reveal a binding in the test system. Aspects of the reactive structure on β_2-microglobulin are currently being explored.

In the present investigations we have defined a surface protein structure on group A, C, and G streptococci with reactivity for aggregated β_2-microglobulin. This interaction adds a new type of mammalian-microbial short circuit to some other types of interactions known to occur with group A, C, and G streptococci. Fibrinogen will bind to group A streptococci (20), to their M-protein (9), and also to group C and G streptococci (Kronvall et al., manuscript in preparation). IgG will bind to group A streptococci (Fc-binding protein type II) and to group C and G streptococci (Fc-binding protein type III) (10, 14).

The existence of a range of possible interactions between mammalian proteins and bacterial surface structures suggests an important role of short circuits in host-parasite relations (12).

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LITERATURE CITED

1. **Anderson, C. L., R. T. Kubo, and H. M. Grey.** 1975. Studies on the cytophilic properties of human β_2-microglobulin. J. Immunol. **114**:997-1000.
2. **Avrameas, S.** 1969. Coupling of enzymes to proteins with glutaraldehyde. Use of the conjugates for the detection of antigens and antibodies. Immunochemistry **6**:43-52.
3. **Berggård, I.** 1974. Isolation and characteristics of a rabbit β_2-microglobulin: comparison with human β_2-microglobulin. Biochem. Biophys. Res. Commun. **57**: 1159-1165.
4. **Berggård, I., and A. G. Bearn.** 1968. Isolation and properties of a low molecular weight β_2-microglobulin occurring in human biological fluids. J. Biol. Chem. **243**:4095-4103.
5. **Björck, L., R. Cigén, B. Berggård, B. Löw, and I. Berggård.** 1977. Relationships between β_2-microglobulin and alloantigens coded for by the major histocompatibility complexes of the rabbit and the guinea pig. Scand. J. Immunol. **6**:1063-1069.
6. **Ellen, R. P., and R. J. Gibbons.** 1972. M protein-associated adherence of *Streptococcus pyogenes* to epithelial surfaces: prerequisite for virulence. Infect. Immun. **5**:826-830.
7. **Forsgren, A., and J. Sjöquist.** 1966. Protein A from *S. aureus*. I. Pseudoimmune reaction with human γ-globulin. J. Immunol. **97**:822-827.
8. **Hunter, W. H., and F. C. Greenwood.** 1962. Preparation of iodine-131 labelled human growth hormone of high specific activity. Nature (London) **194**:495-496.
9. **Kantor, F. S., and R. M. Cole.** 1959. A fibrinogen precipitating factor (FPF) of group A streptococci. Proc. Soc. Exp. Biol. Med. **102**:146-150.
10. **Kronvall, G.** 1973. A surface component in group A, C, and G streptococci with non-immune reactivity for immunoglobulin G. J. Immunol. **111**:1401-1406.
11. **Kronvall, G., and E. Myhre.** 1977. Differential staining of bacteria in clinical specimens using acridine orange buffered at low pH. Acta Pathol. Microbiol. Scand. Sect. B **85**:249-254.
12. **Kronvall, G., and R. C. Williams, Jr.** 1969. Immunologic "short circuits." Ann. Intern. Med. **70**:1043-1045.
13. **McConahey, P. J., and F. J. Dixon.** 1966. A method of trace iodination of proteins for immunologic studies. Int. Arch. Allergy Appl. Immunol. **29**:185-189.
14. **Myhre, E. B., and G. Kronvall.** 1977. Heterogeneity of nonimmune immunoglobulin Fc reactivity among gram-positive cocci: description of three major types of receptors for human immunoglobulin G. Infect. Immun. **17**:475-482.
15. **Painter, R. H., D. Yasmeen, S. N. Assimeh, and M. D. Poulik.** 1974. Complement fixing and macrophage opsonizing activities associated with β_2-microglobulin. Immunol. Commun. **3**:19-34.

16. **Peterson, P. A., B. A. Cunningham, I. Berggård, and G. M. Edelman.** 1972. β_2-microglobulin—a free immunoglobulin domain. Proc. Natl. Acad. Sci. U.S.A. **69**:1697-1701.
17. **Peterson, P. A., L. Rask, K. Sege, L. Klareskog, H. Anundi, and L. Östberg.** 1975. Evolutionary relationship between immunoglobulins and transplantation antigens. Proc. Natl. Acad. Sci. U.S.A. **72**:1612-1616.
18. **Smithies, O., and M. D. Poulik.** 1972. Initiation of protein synthesis at an unusual position in an immunoglobulin gene. Science **175**:187-189.
19. **Terhorst, R. Robb, C. Jones, and J. L. Strominger.** 1977. Further structural studies of the heavy chain of HLA antigens and its similarity to immunoglobulins. Proc. Natl. Acad. Sci. U.S.A. **74**:4002-4006.
20. **Tillett, W. S., and R. L. Garner.** 1934. The agglutination of hemolytic streptococci by plasma and fibrinogen. Bull. Johns Hopkins Hosp. **54**:145.