SURFACE RECEPTORS FOR HUMAN SERUM ALBUMIN IN PEPTOCOCCUS MAGNUS STRAINS

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Summary. Eighty-one bacterial strains representing 16 anaerobic species were tested in a sensitive binding assay for uptake of ¹²⁵I-labelled human serum proteins. Fifteen of 36 Peptococcus magnus strains (42%) bound significant amounts of human serum albumin (HSA). None of the other bacterial species showed any affinity for HSA. All strains studied were incapable of uptake of human fibrinogen, fibronectin, haptoglobin or aggregated β₂-microglobulin. P. magnus strain Ra 4 was tested for binding of purified serum albumin from 11 animal species, and showed a binding profile similar to human group-C and -G streptococci, but different from Streptococcus pyogenes, Strep. zooepidemicus and Strep. dysgalactiae. Kinetic experiments showed that albumin binding was a rapid displaceable, time-dependent process, that could take place over a wide range of pH or salt concentrations. The albumin-binding component of P. magnus strain Ra 4 was resistant to heat and to periodate treatment, but sensitive to proteolytic enzymes.

Introduction

Previous studies have shown that group-A, -C and -G streptococci can interact with several human serum proteins that include immunoglobulin (Kronvall, 1973), serum albumin (Myhre and Kronvall, 1980), fibrinogen (Kronvall, Schönbeck and Myhre, 1979), fibronectin (Myhre and Kuusela, 1983), haptoglobin (Köhler and Prokop, 1978) and aggregated β₂-microglobulin (Kronvall et al., 1978). These reactivities are mediated by separate sets of binding sites present on the streptococcal cell surface. The various types of receptors have not been identified structurally and are currently defined by their selective specificity for the corresponding serum protein. Binding of host protein to microorganisms is a host-parasite interaction and may, therefore, be significant in the pathogenesis of infections caused by these organisms. Group-A, -C and -G streptococci grown in anaerobic conditions also interact with human IgG, serum albumin and fibrinogen (unpublished findings). It was therefore considered relevant to examine strictly anaerobic bacteria for binding of human serum proteins.

MATERIALS AND METHODS

Bacterial strains. Eighty-one anaerobic strains listed in the table were included in the study. Seventy-four strains were clinical isolates collected from specimens received at the Clinical Microbiology Laboratory, University Hospital, Lund, Sweden; seven strains were reference strains kindly provided by Professor C.E. Nord, National Bacteriological Laboratory, Stockholm, Sweden. The strains were identified by morphology, biochemical tests and gas-chromatography (Holdeman, Cato and Moore, 1977). The albumin-reactive group-A, -C and -G streptococci have been described in an earlier report (Myhre and Kronvall, 1980).

Growth conditions. Haemolytic streptococci were grown aerobically in Todd-Hewitt broth and anaerobically in chopped-meat-glucose broth (CMG). Initial studies were performed with anaerobic bacteria grown in CMG and in peptone-yeast-glucose broth. There was no noticeable difference in binding reactivity between organisms grown in the two media. Further work was performed with bacteria cultured in CMG broth because most strains grew better in this medium. After incubation for 36 h at 37°C, the solid meat particles were removed from the cultures by centrifugation at 200g for 10 min. The supernate was transferred to a new tube and the organisms pelleted by centrifugation at 2000 g for 10 min. The bacteria were washed and suspended in buffer to a concentration of 10⁹/ml as previously described (Myhre and Kronvall, 1980).

Human proteins. Serum albumins from various animals (see figure) were prepared (Myhre and Kronvall, 1980) and albumin concentrations were measured spectrophotometrically (Peters, 1975). Mouse, goat and pigeon albumin were from the Sigma Chemical Company (St Louis, MO, USA) and bovine serum albumin and human fibrinogen from AB Kabi (Stockholm, Sweden). Haptoglobin was from Behringwerke AG (Marburg, GFR) and aggregated β₂-microglobulin (Kronvall et al., 1978) was a gift from Dr L. Björck, University of Lund, Sweden. Fibronectin was purified from human plasma (Myhre and Kuusela, 1983). The purity of human serum albumin, fibrinogen and haptoglobin was confirmed by crossed immunoelectrophoresis against specific rabbit antisera (Dakopatts AB, Copenhagen, Denmark).

Radiolabelling. Purified human proteins were labelled with ¹²⁵I (Radiochemical Centre, Amersham, UK) by a modified lactoperoxidase method (Myhre and Kuusela, 1983).

Binding assay. Quantitative binding experiments were performed in phosphate buffered saline with Tween 20·0·05% (PBSA-Tween) as previously described (Myhre and Kronvall, 1980). Experiments were also performed with bacteria suspended in 0·1 M acetate (pH 3·0–5·0), 0·1 M phosphate (pH 6·0–7·0) and 0·1 M Tris-HCl (pH 8·0–9·0) buffers. The rate of binding of human serum albumin (HSA) to 10⁸ bacteria was determined by measuring the uptake of 20 ng of labelled HSA at intervals from 10 s to 60 min. The dissociation process was studied by suspending peptococci pretreated with 500 ng of labelled HSA in 500 μl of PBSA-Tween buffer containing unlabelled HSA 10 mg/ml. After 30 and 60 min, 2 ml of PBSA-Tween buffer was added, the bacteria sedimented by centrifugation and the amount of radioactivity still bound to the bacteria determined.

Inhibition experiments were performed by adding increasing amounts (1–1000 μg) of HSA, polyclonal IgG and fibrinogen, and various dilutions of normal human serum (1 in 10–1 in 10000) to 20 ng of labelled HSA. The samples were then incubated with 10⁸ bacteria in a final volume of 500 μl of PBSA-Tween buffer and the uptake of radioactive material determined as described above.

Immunofluorescence microscopy. Albumin-positive and -negative P. magnus strains (10⁸ bacteria) were incubated in 500 μl of normal human serum diluted 1 in 10 in PBSA-Tween buffer. The bacteria were washed and reincubated in PBSA-Tween buffer containing monospecific rabbit antibodies to HSA (Dakopatts AB). The organisms were then washed and stained with FITC-labelled swine antirabbit IgG antibodies (Dakopatts AB). Samples were examined with a Leitz Ortholux II immunofluorescence microscope. Each incubation step was done at 37°C for 30 min.

Elution of bacteria-associated HSA. Peptococci with receptor-associated labelled HSA were suspended in 2 ml of potassium thiocyanate (KSCN) (1–3 M), 2 ml of urea (1–6 M) or 2 ml of glycine-HCl (0·1 M, pH 3·0) solution. After 30 min at room temperature the bacteria were deposited by centrifugation and the radioactivity of the pellet measured.
**SERUM ALBUMIN RECEPTORS IN P. MAGNUS**

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**Source of serum albumin**

Figure—Capacity of (a) *P. magnus*, (b) *Strep. equisimilis*, (c) human group-G streptococcus, (d) bovine group-G streptococcus, (e) *Strep. pyogenes*, (f) *Strep. zooepidemicus* and (g) *Strep. dysgalactiae* to bind radiolabelled serum albumin of human and of animal origin. Binding profiles were recorded with 20 ng of albumin and $10^8$ bacteria in PBSA-Tween buffer.

**Enzymatic and chemical treatment of bacteria.** Pepsin and trypsin digestion was performed as previously described (Myhre and Kronvall, 1980). Papain digestion was done at 37°C with 100 μg of enzyme and $10^8$ bacteria suspended in 1 ml of 0·1 M phosphate buffer, pH 7·0, with 10 mM EDTA-Na₂. After 1 h, iodoacetamide was added to give a concentration of 20 mM and the cells were washed, resuspended and tested in direct binding experiments. Bacteria were treated with periodate by suspending the organisms in a 10 mM sodium metaperiodate solution (Ofek, Mirelman and Sharon, 1977). After incubation for 10 min at room temperature, the bacteria were washed and suspended in PBSA-Tween buffer.

**RESULTS**

**Screening of anaerobic bacteria for albumin reactivity**

A total of 81 bacterial strains representing 16 anaerobic species were tested and 15 of 36 *P. magnus* strains bound significant amounts (21–85%) of HSA (table). None of the other bacterial species studied showed any affinity for HSA. The uptake of human fibrinogen, fibronectin, haptoglobin and aggregated β₂-microglobulin to the anaerobic strains included in the study was < 5%, probably reflecting non-specific binding.

Four *P. magnus* strains found to differ in reactivity were retested in quantitative
TABLE

Percentage uptake of $^{125I}$-labelled human serum albumin (HSA) by various bacterial species

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of strains tested</th>
<th>Reference strain included*</th>
<th>Percentage uptake of labelled HSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptococcus magnus</td>
<td>36</td>
<td>ATCC 29328</td>
<td>3–85†</td>
</tr>
<tr>
<td>Peptococcus asaccharolyticus</td>
<td>2</td>
<td>ATCC 14963</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Peptococcus prevotii</td>
<td>1</td>
<td>ATCC 9321</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Peptostreptococcus anaerobius</td>
<td>3</td>
<td>ATCC 14955</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Streptococcus constellatus</td>
<td>1</td>
<td>ATCC 27823</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Streptococcus intermedius</td>
<td>1</td>
<td>ATCC 27335</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Streptococcus morbillorum</td>
<td>2</td>
<td>ATCC 27824</td>
<td>&lt;5</td>
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<tr>
<td>Bacteroides fragilis</td>
<td>14</td>
<td>ATCC 29328</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Bacteroides ovatus</td>
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<td>ATCC 29328</td>
<td>&lt;5</td>
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<td>Bacteroides thetaiotaomicron</td>
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<td>&lt;5</td>
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<td>Bacteroides vulgatus</td>
<td>1</td>
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<td>&lt;5</td>
</tr>
<tr>
<td>Bacteroides ureolyticus</td>
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<td>ATCC 29328</td>
<td>&lt;5</td>
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<td>Clostridium perfringens</td>
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<td>&lt;5</td>
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<td>Clostridium difficile</td>
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<td>&lt;5</td>
</tr>
<tr>
<td>Clostridium sporogenes</td>
<td>1</td>
<td>ATCC 29328</td>
<td>&lt;5</td>
</tr>
</tbody>
</table>

* American type culture collection strains.
† 15 strains bound 21–85% (X = 56%); 21 strains bound <8%.

binding assays with various amounts of HSA. A correlation was seen between the relative uptake expressed as a percentage and the absolute binding capacity measured in µg. Two of the strains that had shown a binding level of ≈80% bound approximately 1 µg of HSA in tests performed with $10^8$ organisms. The two other strains found in the initial experiments to bind 30–40% of the labelled material bound only 0.2–0.3 µg of HSA when tested in parallel experiments with a final HSA concentration of 10 µg/ml. These quantities represent the total HSA binding capacity of the strains because an increase in HSA concentration to 100 µg/ml did not increase the amount of HSA bound. *P. magnus* strain Ra 4 showed consistently high binding levels and subsequent experiments were performed with this strain.

Unlabelled HSA and normal human serum inhibited, in a dose-dependent way, the uptake of isotope-labelled HSA to *P. magnus* (data not shown). Complete inhibition of the binding to $10^8$ organisms suspended in 500 µl of buffer was achieved with 20 µg of HSA and with 20 µl of human serum diluted 1 in 2000. Human polyclonal IgG and fibrinogen, even at concentrations of 5 mg/ml did not inhibit the HSA uptake.

Albumin-reactive *P. magnus* strains exposed to normal human serum showed a uniform surface fluorescence when stained with HSA-specific reagents and examined by indirect immunofluorescence. Albumin-negative control organisms showed no fluorescence indicating that albumin-binding peptococci can absorb HSA in the presence of other serum proteins.

**Binding of serum albumins from non-human species**

A panel of 11 purified isotope-labelled albumins of animal origin were tested for quantitative uptake to *P. magnus* and group-A, -C and -G streptococci, species known to interact with HSA. *P. magnus* strain Ra 4 (figure) demonstrated a binding profile characterised by high uptake of human, mouse and cat albumin, and low but definite
uptake of guinea pig, dog and horse albumin. Rabbit, pig, sheep, goat, cow and pigeon albumin showed only a background binding of <10%. Human group-C streptococci (Strep. equisimilis) and human group-G streptococci showed very similar binding profiles (figure). Bovine group-G streptococci demonstrated a slightly different profile with somewhat higher uptake of horse and pig albumin (figure). Strep. pyogenes, Strep. zooepidemicus and Strep. dysgalactiae showed three entirely different binding patterns, each distinct from that of P. magnus (figure). Streptococci grown aerobically and anaerobically did not differ in binding pattern.

Kinetic experiments

Albumin binding with P. magnus strain Ra 4 was a time-dependent process with a rapid association phase similar to that found with a human group-G streptococcus (Myhre and Kronvall, 1980). After 10, 30 and 60 s, uptake levels of 21, 42 and 55% were noted, but between 60 s and 60 min there was little increase in uptake. Exposure of P. magnus cells with bound labelled HSA to a large excess of free unlabelled HSA displaced 15% of the labelled HSA in 60 min.

Characterisation of the HSA-binding structure

Heating of P. magnus organisms to 80°C for 5 min did not affect the reactivity measured in direct binding assays. The albumin-binding site was sensitive to digestion with pepsin and trypsin. Complete loss of binding reactivity was observed with as little as 2 μg of pepsin and 10 μg of trypsin. Digestion with 100 μg of papain reduced the binding from 80% to 5%. Bacterial cells treated with sodium metaperiodate, a reagent known to cleave C-C bonds between vincinal hydroxyl groups of sugars, did not affect the uptake of HSA.

The effect of pH on the HSA uptake was assessed with peptococci suspended in buffers with pH values from 3.0 to 9.0. Unspecific background binding was measured with trypsin-treated bacteria. Untreated cells showed binding over the entire pH range tested, with the highest uptake between pH 5.0 and 8.0. Trypsin-treated bacteria bound HSA only at pH values below 6.0.

High uptake was seen in 0.01 M phosphate buffer, pH 7, at all concentrations of NaCl between 0.15 and 0.6 M but a small increase in binding activity was observed at NaCl concentrations below 0.15 M. Tween 20, even at a concentration of 1% did not reduce the binding capacity of the organisms. HSA was firmly bound to peptococci and <5% of bound albumin could be released by repeated washes in PBSA-Tween buffer, but exposure of P. magnus organisms preincubated with labelled HSA to increasing concentrations of KSCN resulted in a dose-dependent release of albumin from the surface receptors. Glycine-HCl (0.1 M, pH 3.0) caused the dissociation of of the labelled material from the cells.

DISCUSSION

Albumin reactivity, the specific interaction between serum albumin and structures on the bacterial cell surface, has been described previously in streptococci of Lancefield's serogroups A, C and G (Kronvall et al., 1979, Myhre and Kronvall, 1980).
The demonstration, in the present investigation, of albumin binding to *P. magnus*, but not to any of the other 16 anaerobic species studied, suggests some relationship between this species and group-A, -C and -G streptococci. The various types of HSA receptors may have a common origin implying a phylogenetic relationship between *P. magnus* and group-A, -C and -G streptococci. The binding experiments with a panel of animal albumins provided further evidence for a close relationship between *P. magnus* and human group-C and -G streptococci, and confirm the existence of distinct types of albumin receptors described previously by other authors (Widebäck and Kronvall, 1982; Widebäck, Seal and Kronvall, 1982; Widebäck, Havlicek and Kronvall, 1983).

Human polyclonal IgG and fibrinogen did not affect the uptake of isotope-labelled HSA to bacteria. Pooled normal human serum inhibited the binding of $^{125}$I-HSA to the same degree as did purified HSA at a concentration corresponding to the HSA concentration in serum. These observations indicate that the albumin reactivity is a specific interaction and that serum proteins other than HSA are not capable of binding to the albumin receptor.

The rapid uptake of $^{125}$I-HSA to intact organisms suggests that the binding sites are exposed at the periphery of the peptococcus cell. Although the receptor was not defined structurally, its characteristics, i.e., sensitivity to proteolytic enzymes and resistance to heat and periodate treatment, are identical with those reported for the HSA-receptors in β-haemolytic streptococci (Myhre and Kronvall, 1980).

The albumin-reactive *P. magnus* strains identified in the present investigation were, with the exception of a single reference strain, fresh human isolates recovered from clinical specimens. Although HSA binding was studied *in vitro*, these strains may also bind albumin *in vivo* in man. Immunofluorescence studies of *P. magnus* exposed to human serum showed that the bacteria were uniformly coated with HSA. Complete saturation of the albumin receptors, defined by complete inhibition of the $^{125}$I-HSA uptake by unlabelled HSA, was seen at an albumin concentration as low as 40 μg/ml, i.e., at a level one thousand times lower than the average serum concentration of 40 g/L. Albumin is present extravascularly in most tissues and presumably on mucosal surfaces during an inflammatory reaction. The present studies showed that albumin binding can take place over a wide pH range and at various salt concentrations, so that *P. magnus* organisms may rapidly become coated with HSA once they invade the human body. The clinical significance of these findings requires further study.

The author is indebted to Mrs E. Holst for help in collecting and growing anaerobic bacterial strains and to Miss B.-M. Kjellberg for skilled technical assistance. This project was supported by a grant from the A. Österlund Foundation.

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