Streptococcus uberis Resists the Bactericidal Action of Bovine Neutrophils Despite the Presence of Bound Immunoglobulin

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***Streptococcus uberis* 0140J was more resistant to the bactericidal action of bovine neutrophils after growth in chemically defined medium (CDM) supplemented with casein hydrolysate than when grown in CDM alone. Neither adult bovine serum obtained prior to vaccination nor hyperimmune serum raised against this bacterium was capable of acting as an effective opsonin towards *S. uberis* grown in the presence of casein hydrolysate. There was no detectable difference in the ability of bacteria grown in either CDM or CDM supplemented with casein hydrolysate to bind immunoglobulin G1 (IgG1), IgG2, or IgM from either hyperimmune serum or preparations of immunoglobulin from the same serum. Bacteria of both the phagocytosis-resistant and phagocytosis-sensitive phenotypes presented the same amount of IgG2 Fc terminus on their surfaces. It is concluded that the inducible resistance of *S. uberis* to bactericidal action of bovine neutrophils is not mediated by inhibition of antibody binding.**

Streptococcus uberis is a common cause of bovine mastitis and is responsible for about 20% of all clinical cases of mastitis in the United Kingdom (2). Phagocytosis by neutrophils is a crucial process in the elimination of *Escherichia coli* (6) and *Staphylococcus aureus* (18) from the bovine mammary gland. This has not been established for the control of intramammary infection with *S. uberis*, although it has been shown that a phagocytosis-resistant strain of *S. uberis* (15) was more infective for the lactating mammary gland (4) than a strain which was easily phagocytosed (15).

The resistance of *S. uberis* to the bactericidal action of bovine neutrophils in vitro is induced by growth of the bacterium in media containing casein-derived amino acids and peptides (14) which may mimic the state within the lactating mammary gland during infection. Phagocytosis-resistant *S. uberis* possesses a hyaluronic acid capsule, whereas phagocytosis-sensitive *S. uberis* does not (14). However, the role of this surface layer in the resistance of *S. uberis* to phagocytosis is unproven.

Two general mechanisms have been proposed to account for the role of capsular polysaccharide in resistance to phagocytosis. First, the capsule may prevent the binding of opsonic factors such as antibody or complement (8). Alternatively, it may be permeable to opsonic factors which bind beneath the capsular surface such that the capsule presents a barrier that prevents subsequent contact between opsonin ligands and their receptors on the phagocytic cell (10). The capsule is therefore thought to act as a physical barrier to the efficient attachment of either opsonin or the phagocyte and the bacterium.

The ability to change the resistance of *S. uberis* to phagocytosis and killing by neutrophils by altering growth conditions has allowed the comparison of phagocytosis-resistant and -sensitive phenotypes of the organism. In this communication, we report an investigation of the ability of phagocytosis-resistant and -sensitive phenotypes of the same strain of *S. uberis* to bind immunoglobulin isotypes from bovine serum.

MATERIALS AND METHODS

Bacterial growth. Cultures of *S. uberis* 0140J were routinely grown in 10 ml of Todd-Hewitt broth at 37°C, washed once in phosphate-buffered saline (PBS; pH 7.2), and resuspended in 1.0 ml of the same buffer. A chemically defined medium (CDM; Table 1) containing 1.0% (wt/vol) glucose, and 1% (wt/vol) casein hydrolysate where indicated, was inoculated with 100 µl of this suspension per 20 ml of medium and incubated at 37°C; bacteria were harvested during the exponential growth phase at an optical density (at 550 nm) of around 1.0.

Production of hyperimmune bovine serum. A nonlactating pregnant Friesian dairy cow from one of the herds at the Institute for Animal Health (Compton, Berks, United Kingdom) was vaccinated subcutaneously with a washed suspension of formalin-killed (1% [vol/vol] formalin in PBS at 4°C for 18 h) *S. uberis* (grown in CDM containing 1% [wt/vol] casein hydrolysate) in Freund's incomplete adjuvant (Difco). Each dose consisted of 1 ml of the bacterial suspension (equivalent to 10¹⁰ CFU), 1 ml of Freund's incomplete adjuvant, and 2 ml of Tween 20 (1% [wt/vol] in PBS). Ten vaccinations were administered at 14-day intervals, and serum was produced from blood collected prior to the first injection and 13 days after subsequent injections.

Isolation of immunoglobulin from hyperimmune serum. Hyperimmune bovine serum was produced from blood collected 13 days after the 10th immunization. Immunoglobulins G and M (IgG and IgM) were separated by gel filtration of 6-ml portions of bovine serum through a column of Sephadex G-200 (2.5 cm [diameter] by 100 cm) equilibrated with PBS (pH 7.2) at an upward flow rate of 15 ml/h. The eluent was collected as 12-ml fractions, and protein was detected by measuring their *A*₂₈₀.

The excluded peak was collected, concentrated by ultrafiltration (10-kDa exclusion limit), and subjected to a second identical gel filtration. The major peak was collected and used as the IgM preparation.

The second protein peak obtained by gel filtration of bovine serum contained both IgG1 and IgG2. These isotypes were purified by affinity chromatography using immobilized protein A. IgG1 and IgG2 from the gel filtration column were applied

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TABLE 1. Composition of the CDM used for the cultivation of *S. uberis* (14)

Component	Concn (mg/liter)
Buffers and salts	
KH ₂ PO ₄	440
K ₂ HPO ₄	300
Na ₂ HPO ₄	3,150
NaH ₂ PO ₄	2,050
(NH ₄) ₂ SO ₄	600
NaCl.....	10
NaHCO ₃	2,000
Na ₂ CO ₃	2,000
Sodium acetate.....	6,000
Trisodium citrate.....	225
Amino acids	
Glutamine.....	5
L-Glutamic acid.....	300
L-Arginine.....	200
L-Proline.....	200
L-Hydroxyproline.....	200
L-Phenylalanine.....	100
L-Tryptophan.....	200
L-Tyrosine.....	200
L-Aspartic acid.....	100
L-Lysine.....	110
L-Methionine.....	100
L-Threonine.....	100
L-Isoleucine.....	100
L-Serine.....	100
L-Glycine.....	200
L-Cysteine.....	100
L-Cystine.....	200
DL-Alanine.....	200
L-Leucine.....	100
L-Valine.....	100
L-Histidine.....	200
Vitamins	
Riboflavin.....	0.4
D-Biotin.....	0.01
Folic acid.....	0.1
Calcium pantothenate.....	0.8
p-Amino benzoic acid.....	0.1
Thiamine hydrochloride.....	0.4
Niacinamide.....	2.0
Pyridoxamine hydrochloride.....	0.8
Nucleic acid bases	
Adenine.....	35
Guanine.....	27
Uracil.....	30
Trace elements	
MgSO ₄ · 7H ₂ O.....	200
FeSO ₄ · 7H ₂ O.....	10
MnSO ₄ · 4H ₂ O.....	10

to a column (4 ml) containing 80 mg of protein A immobilized on Sepharose CL-4B (Sigma). Unbound protein was reapplied to the column until no further reduction in the A_{280} of the eluent was detectable. The unbound fraction contained only IgG1. The column containing bound IgG2 was washed with 10 column volumes of 0.2 M sodium phosphate buffer (pH 7.2), and the IgG2 was eluted with 0.2 M glycine-HCl buffer (pH 2.8) and collected as 1-ml fractions into 50 μ l of neutralizing buffer (1.0 M Tris-HCl [pH 9.0]).

All immunoglobulin preparations were dialyzed against PBS (pH 7.2) prior to use, and their purities were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Cross-contamination of each isotype was deter-

mined by enzyme-linked immunosorbent assay (ELISA) using bovine isotype-specific murine monoclonal antibodies as described below.

ELISA for the determination of immunoglobulin isotype. Aliquots (100 μ l) of each immunoglobulin preparation (10 μ g/ml of carbonate buffer [0.05 M]; pH 9.6) were placed in wells of a flat-bottom microtiter plate (ICN Flow Laboratories) and incubated at 4°C for 18 h. Unbound protein was removed by washing in excess ELISA buffer (PBS containing 0.05% [vol/vol] Tween 20) and blocked with skim milk (1% [wt/vol] Marvel in ELISA buffer) for 30 min at room temperature. Specific immunoglobulin isotypes were detected by the addition of 100 μ l of mouse anti-bovine isotype-specific monoclonal antibodies and then incubation at room temperature for 1 h. Incubation under similar conditions with biotinylated anti-mouse antisera (Amersham International PLC) and streptavidin conjugated to horseradish peroxidase (Amersham International PLC) followed. All reagents were used at saturating concentrations, and plates were washed with excess ELISA buffer to remove unbound reagents between each incubation.

Colorimetric detection of antibody was achieved by the addition of 75 μ l of *O*-phenylenediamine (0.34 mg/ml) in citrate buffer (0.1 M citric acid, 0.2 M Na₂HPO₄ [pH 5.5]) containing hydrogen peroxide (0.03%). Color development was allowed to proceed for approximately 5 min and then stopped by the addition of 150 μ l of H₂SO₄ (1.0 M). The A_{492} of each dilution was measured by using an Anthos Elisa Plate Reader (Denley Instruments Ltd.) and compared with that of a similarly treated control suspension to which bovine immunoglobulin preparations had not been added.

Murine hybridoma cell lines B37, B192, and B67, producing monoclonal antibodies specific for bovine IgG1, IgG2, and IgM, respectively, were a gift to the Institute for Animal Health from C. Stokes, University of Bristol, Bristol, United Kingdom.

Measurement of *S. uberis*-specific immunoglobulin by ELISA. Bacteria were prepared in CDM supplemented with casein hydrolysate as described above and stored in aliquots at -70°C. Prior to use, aliquots were diluted in carbonate buffer (0.05 M, pH 9.6) to give 10⁶ CFU/ml. Wells of flat-bottom microtiter trays (ICN Flow Laboratories) which had been pretreated with 100 μ l of 0.025% (vol/vol) glutaraldehyde in 0.05 M carbonate buffer (pH 9.6) for 1 h at room temperature were coated with 100 μ l of the bacterial suspension and incubated overnight at 4°C. The plates were washed thoroughly with ELISA buffer and blocked with skim milk (1% [wt/vol] Marvel in ELISA buffer) for 30 min at room temperature. Samples of serum were diluted in ELISA buffer, added to the appropriate wells, and incubated for 1 h at room temperature. Bound antibody was detected by using monoclonal antibodies specific for bovine IgG1, IgG2, or IgM (as described above). The titer was calculated as the highest dilution of a sample with an optical density (at 492 nm) which was 1.5 times that obtained in the absence of bovine immunoglobulin (5).

Detection of immunoglobulin bound to *S. uberis*. Bacterial suspensions were harvested by centrifugation (5,000 $\times$ g, 15 min) and washed in ELISA buffer. The suspension was adjusted to an optical density (at 550 nm) of 0.25, and 750 μ l was mixed with 45 μ l of either bovine serum or isolated immunoglobulin (0.9 mg of IgG1 per ml, 2.1 mg of IgG2 per ml, and 1.1 mg of IgM per ml). Bacteria were incubated in the presence of bovine serum or immunoglobulin for 1 h at 37°C.

Bound immunoglobulin was detected by harvesting the bacteria by centrifugation, washing them three times in an equal volume of ELISA buffer, and incubating them at room

temperature for 1 h with either rabbit anti-bovine globulin conjugated to horseradish peroxidase (ICN Flow Laboratories) or monoclonal antibodies specific for individual immunoglobulin isotypes (as described above). To determine the orientation and integrity of bound IgG2, specific detection of the Fc terminus of this isotype was also performed by using the same system to which 45 μ l of a 0.5-mg/ml concentration of protein A-horseradish peroxidase conjugate (Sigma) was used as the detecting reagent.

Bacteria were finally harvested, washed in ELISA buffer, and resuspended in 500 μ l of ELISA buffer. The amount of bound globulin was determined by twofold dilution of 75- μ l portions of the bacterial suspension in ELISA buffer in a flat-bottom microtiter tray (ICN Flow Laboratories), and colorimetric detection was achieved as described previously.

Isolation of bovine neutrophils. Neutrophils were isolated from venous blood obtained from a lactating adult Friesian dairy cow. Blood was collected into 0.1 volume of 13.2 mM sodium phosphate buffer containing 12 mM sodium chloride and 1.5% (wt/vol) EDTA. The erythrocytes and neutrophils were sedimented by centrifugation (1,000 $\times$ g, 15 min), the plasma and buffy coat layer were removed, and the sedimented neutrophils and erythrocytes were weighed. Erythrocytes were lysed by the addition of 4 ml of distilled water per g of sedimented cells. After 45 s, the isotonicity of the cell suspension was restored by the addition of 2 ml of 2.7% (wt/vol) sodium chloride per g of sedimented cells. Neutrophils were harvested by centrifugation (200 $\times$ g, 5 min), washed three times in PBS, and finally resuspended at a density of 10^7 cells per ml for use in the bactericidal assay. The purity and viability of neutrophil suspensions were achieved by microscopic examination.

Bactericidal assay. The resistance of the bacteria to phagocytosis was determined by the method of Leigh and Field (14). Briefly, 0.1 ml of a bacterial suspension (6×10^6 CFU/ml) was mixed with 0.2 ml of a suspension of bovine neutrophils (10^7 cells per ml) and 0.3 ml of either prevaccination or hyperimmune bovine serum (20% [vol/vol] in Hanks balanced salt solution [Gibco]). The mixtures were rolled at 120 rpm for 90 min on a Coulter roller (Coulter Electronics) at 37°C. The number of viable bacteria was determined by dilution in isotonic saline and incubating three 20- μ l samples of each dilution on blood agar containing 1% (wt/vol) esculin at 37°C for 18 h. The percentage survival was determined by comparison of the bacterial colony counts obtained from the determinations described above with those obtained from an identically treated control to which Hanks balanced salt solution without neutrophils had been added.

RESULTS

The level of *S. uberis* antibody in bovine serum increased after vaccination with formalin-killed bacterial cells (Fig. 1). Both IgG2 and IgM showed maximal responses after four vaccinations which represented 5-fold and 20-fold increases over the prevaccination values, respectively. The level of *S. uberis*-specific IgG1 increased after the second and third vaccinations. Samples obtained after the fourth vaccination showed a fall in the level of *S. uberis*-specific IgG1 in serum. This corresponded to calving and the early stages of lactation in this animal. At this time, IgG1 present in plasma is actively transported into the colostrum and early lactation milk (12). Several weeks into lactation, at which time IgG1 is no longer removed from the plasma into the milk in such quantity, the amount of *S. uberis*-specific IgG1 in the serum rose to levels 20-fold greater than that seen prevaccination (Fig. 1). After

FIG. 1. Change in *S. uberis*-specific antibody titer of bovine serum after subcutaneous vaccination with formalin-killed *S. uberis*. Titers are shown for IgG1 (circles), IgG2 (squares), and IgM (triangles). Specific immunoglobulin was detected by using isotype-specific murine monoclonal antibodies.

eight immunizations, no further increase in *S. uberis*-specific immunoglobulin, of any isotype, could be detected following subsequent administration of antigen. Therefore, hyperimmune serum was prepared from blood collected 13 days after the 10th immunization and used for the preparation of individual immunoglobulin isotypes and as a source of opsonin in the bactericidal assay.

The viability of neutrophils obtained from venous blood, determined by the exclusion of trypan blue, was in excess of 97%, and the purity of such preparations was greater than 90%. The major contaminating cells present in these preparations were monocytes and, occasionally, eosinophils. The presence of these cells had no discernible effect on the bactericidal capability of such preparations for *S. uberis*.

Killing of *S. uberis* by neutrophils was dependent upon the presence of serum (Table 2). The ability of bacteria, grown in chemically defined medium (CDM) alone, to resist the bactericidal action of neutrophils was low in the presence of either prevaccination or hyperimmune serum, indicating that both preparations of serum were capable of opsonizing bacteria grown in this medium. In contrast, the same organism grown in the same medium supplemented with casein hydrolysate showed significantly greater resistance to the bactericidal action of neutrophils, and there was no difference in the ability of

TABLE 2. Percentage survival of *S. uberis* after incubation in the presence of bovine neutrophils and normal adult or hyperimmune serum

Opsonin	% Survival ($\pm$ SEM) ^a of <i>S. uberis</i> grown in:	
	CDM	CDM containing casein hydrolysate ^b
None	131.40 ($\pm$ 1.50)	103.90 ($\pm$ 8.14)
Prevaccination serum ^c	6.77 ($\pm$ 1.84)	49.78 ($\pm$ 3.38)
Hyperimmune serum ^c	5.77 ($\pm$ 2.78)	56.1 ($\pm$ 11.1)
Hyperimmune serum ^d	0.48 ($\pm$ 0.23)	50.1 ($\pm$ 8.98)

^a Means of three triplicate determinations.

^b Casein hydrolysate was added to the medium to a final concentration of 1.0% (wt/vol).

^c Serum was used at a final concentration of 10% (vol/vol).

^d Serum was heated at 56°C for 30 min prior to use at a final concentration of 10% (vol/vol).

either prevaccination or hyperimmune serum to opsonize *S. uberis* cultured in this medium. Depletion of complement from the hyperimmune serum, by heating at 56°C for 30 min, had no significant effect on its ability to opsonize *S. uberis* of either the phagocytosis-resistant or -susceptible phenotype, indicating that complement played little part in the opsonization of *S. uberis*.

Similar observations have been made with several strains of *S. uberis* by using typical adult bovine serum (14) and precolostral calf serum (3a) as a source of opsonin. Despite the boosted levels of *S. uberis*-specific antibody present in the hyperimmune serum (Fig. 1), resistance to phagocytic killing by bovine neutrophils was similar to that previously reported for both the phagocytosis-resistant (those grown in CDM containing casein hydrolysate) and phagocytosis-sensitive (those grown in CDM alone) bacteria. The ability of *S. uberis* to survive intracellular killing was poor. In no instance did the number of viable bacteria obtained from washed and disrupted neutrophils exceed 1% of the total number of viable bacteria remaining at the end of the assay (3a).

Immunoglobulin prepared from the hyperimmune serum contained only single isotypes (IgG1, IgG2, or IgM), as determined by their reaction in an ELISA with isotype-specific monoclonal antibodies. None of the preparations showed any reaction with more than one monoclonal antibody. SDS-PAGE of the IgG1 and IgG2 preparations showed the presence of only two bands at approximately 50 and 25 kDa, which corresponded to the immunoglobulin heavy and light chains, respectively. Because of the nature of the isolation of IgM, this preparation was not pure and, in addition to the immunoglobulin, also contained traces of α -2 macroglobulin.

All of the isolated isotypes bound well to bacteria of both phenotypes (Fig. 2). The resistant bacteria appeared to bind slightly more of each immunoglobulin than the sensitive bacteria, although this difference was not significant. The horseradish peroxidase-rabbit immunoglobulin conjugate used to detect the bound bovine immunoglobulin exhibited nonspecific binding to *S. uberis* in the absence of purified immunoglobulin. In an attempt to overcome this effect, and to determine the ability of the bacteria to bind different immunoglobulin isotypes after incubation in complete bovine serum, monoclonal antibodies specific for bovine IgG1, IgG2, and IgM were used as detecting reagents. After incubation in serum, bacteria of both phenotypes bound equal amounts of each isotype (Fig. 3) and little nonspecific binding of the detecting reagents was observed.

S. uberis of both the resistant and susceptible phenotypes bound equal quantities of the opsonic immunoglobulin (9), IgG2. However, bacteria grown in the presence of casein hydrolysate were not opsonized effectively. To determine whether the resistance of bacteria of this phenotype to the bactericidal action of neutrophils was due to degradation of the immunoglobulin or masking of its Fc terminus, protein A (which shows specificity for the Fc portion of bovine IgG2) conjugated to horseradish peroxidase was also used to detect this isotype on bacterial cells (Fig. 4). Equal quantities of the IgG2 Fc region were detected on bacteria of each phenotype, suggesting that both the phagocytosis-resistant and -susceptible bacteria bound this immunoglobulin in a similar manner.

DISCUSSION

High levels of *S. uberis*-specific antibody, produced by repeated vaccination with formalin-killed bacterial cells, did not promote efficient phagocytosis of *S. uberis* which had been cultivated in the presence of casein hydrolysate. The survival of

FIG. 2. Binding of purified hyperimmune immunoglobulin to *S. uberis*. Bacteria were incubated with either individual immunoglobulin isotypes (circles) or PBS (triangles). Bacteria were grown in either CDM (closed symbols) or CDM containing casein hydrolysate (open symbols). Bound immunoglobulin was detected by using a rabbit anti-bovine immunoglobulin antibody conjugated to horseradish peroxidase.

S. uberis was similar to that seen previously when typical adult serum (14) or precolostral calf serum (3a) was used as a source of opsonin. Since the antibody levels, in all isotypes, obtained in the current investigation could not be increased by further administration of the formalin-killed bacterial cells, it would appear that either this antigen did not contain the necessary epitopes required to generate an effective opsonogenic response to *S. uberis* or that the component responsible for the antiphagocytic effect of the bacterium grown in the presence of casein hydrolysate was not antigenic. The ability of the hyperimmune serum to promote phagocytosis of *S. uberis* was not significantly affected by heating (56°C, 30 min), suggesting that complement components were not involved in the opsonization of this bacterium.

The presence of a hyaluronic acid capsule has been reported on the surface of strains of *S. uberis* which have been cultivated in the presence of casein-derived peptides (14). During the current investigation, the phagocytosis-resistant *S. uberis* was found to bind equal amounts of each isotype of immunoglobulin as the phagocytosis-sensitive *S. uberis*. This indicates that capsule does not prevent antibody from binding to the bacterium and does not behave in a similar manner as the capsules of *E. coli* (8) and *S. aureus* (19), both of which prevented the binding of potentially opsonic ligands to subcapsular components. The possibility that capsule prevents binding between the bacterium and the phagocyte by masking the Fc terminus of bound immunoglobulin, a process known to occur in other bacterial species (10), was not investigated during the present study.

FIG. 3. Binding of individual immunoglobulin isotypes to *S. uberis* after incubation in hyperimmune bovine serum. Bacteria were incubated with either hyperimmune bovine serum (circles) or PBS (triangles). Bacteria were grown in either CDM (closed symbols) or CDM containing casein hydrolysate (open symbols). Bound immunoglobulin was detected by using isotype-specific murine monoclonal antibodies.

In addition to polysaccharide capsules, a variety of bacterial proteins has been reported to be responsible, at least in part, for the resistance of bacteria to phagocytosis. The most notable of these are M protein (13), streptolysins S and O (20), and immunoglobulin-binding proteins (17), all of which can be produced by *Streptococcus pyogenes*, and the immunoglobulin-binding protein, protein A, which is produced by *S. aureus* (3). The exact mechanism by which M protein exerts its antiphagocytic activity is uncertain, but two models have been suggested. The M protein either prevents deposition of complement and/or antibody on the surface of *S. pyogenes* (1) or interferes with actin and myosin contractile fibers of the phagocyte cytoskeleton, thus preventing phagocytosis (16). Since the deposition of potentially opsonic antibody on *S. uberis* of the phagocytosis-resistant phenotype was not prevented and since complement is not involved in the phagocytosis of *S. uberis* of either the resistant or susceptible phenotype, the probability of this bacterium using a mechanism similar to that initially described for M protein on *S. pyogenes* would seem unlikely. Nevertheless, the presence on *S. uberis* of a protein which affects the phagocytic activity of the neutrophil remains an attractive theory, especially since this would function even in the presence of bound, and potentially opsonic, antibody.

The streptolysins S and O are both believed to exert their antiphagocytic effect via cytotoxic activity which leads to morphological changes in the neutrophil (7). Neutrophils incubated with phagocytosis-resistant bacteria, in this study, remained intact (14a) and did not show any gross morphological differences from neutrophils incubated with *S. uberis* of the susceptible phenotype.

Proteins A and G present on *S. aureus* and *S. pyogenes*,

FIG. 4. Detection of the Fc terminus of hyperimmune bovine IgG2. Bacteria were incubated in either hyperimmune bovine serum or purified hyperimmune IgG2 (circles); control suspensions were incubated in PBS (triangles). Bacteria were grown in either CDM (closed symbols) or CDM containing casein hydrolysate (open symbols). Bound immunoglobulin was detected by using protein A conjugated to horseradish peroxidase.

respectively, function by binding immunoglobulin at the Fc terminus (11, 17), thereby rendering this portion of the molecule unavailable for reaction with immunoglobulin receptors present on the neutrophil. It would appear unlikely that such a molecule accounted for the ability of *S. uberis* to resist phagocytosis and killing, since bacteria of both the resistant and susceptible phenotypes presented equal quantities of the Fc portion of the potentially opsonic IgG2 subclass (9) on their surfaces. The disorientation of IgM, which also shows opsonic capability for bovine neutrophils (21), has not been reported previously and was not investigated here.

It is apparent that *S. uberis* cultivated in the presence of casein hydrolysate resists the bactericidal action of bovine neutrophils by a mechanism which neither prevents immunoglobulin binding to the bacterium nor causes inappropriate binding of IgG2 and may be analogous to that suggested for the M protein of *S. pyogenes* (16).

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