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# Localization of Brucella Antigens That Elicit a Humoral Immune Response in *Brucella abortus*-Infected Cattle

GERHARDT G. SCHURIG,[1]* ALASTAIR T. PRINGLE,[2]† AND SYDNEY S. BREESE, JR.[3]

*Division of Pathology and Public Practice, Virginia Maryland Regional College of Veterinary Medicine,[1] and Department of Anaerobic Microbiology,[2] Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061 and Department of Anatomy, Medical Center, University of Virginia, Charlottesville, Virginia 22908[3]*

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Localization of brucella antigens, to which brucella-infected cattle make antibodies, and the surface characteristics of *Brucella abortus* smooth strain 19 and rough strain 45/20 were studied by the use of monospecific antisera in absorption tests, electron microscopy, and electrophoretic mobility of organisms in microelectrophoresis. Antigenic determinants of electrophoretically defined antigen A5 were present on the surface of *B. abortus* rough strain 45/20 organisms, and protein moieties were most probably exposed on the surface of this strain in contrast with smooth strain 19 organisms. Several antigens distinct from the smooth lipopolysaccharide complex, to which brucella-infected cattle make antibodies, were not detected on the surface of smooth organisms. Agglutinating antibodies present in anti-*B. abortus* strain 19 serum did not bind to all areas on the surface of the smooth cells, suggesting the presence of different antigenic moieties on their surface. It is also postulated that the surface of *B. abortus* rough strain 45/20 displays receptors able to strongly bind immunoglobulin molecules, as well as other serum components.

Brucella organisms contain a large number of antigenically distinct components, including the smooth lipopolysaccharide (LPS) of smooth virulent cells and the rough LPS of rough organisms (7, 13). Sera from *Brucella abortus*-infected or vaccinated bovines generally contain antibodies directed against the smooth LPS complex of the outer surface of smooth *B. abortus* cells, as well as antibodies directed against nonsmooth LPS antigens, which can be extracted from rough strain *B. melitensis* B.115, *B. abortus* strain 19 (G. Schurig, unpublished data), and *B. abortus* rough strain 45/20 (13). Seven of these nonsmooth LPS antigens were described by Schurig et al. (13) according to their electrophoretic mobility and reactivity with sera from *B. abortus*-infected and strain 19-vaccinated animals. The location of the nonsmooth LPS antigens within the brucella organisms, as well as the role of the immune response to them, is unknown.

It is generally assumed that an immune response to antigens superficially located on invading microorganisms will be of more importance to the host in its effort to eliminate the pathogen as a response to internal antigens.

† Present address: Department of Microbiology and the Molecular Biology Institute, University of California, Los Angeles, CA 90024.

The present study describes the localization, visualization, and distribution of the smooth LPS complex on the surface of *B. abortus* strain 19, as well as the localization of one nonsmooth LPS antigen on the surface of *B. abortus* strain 45/20 organisms. It also describes the electrophoretic mobility of smooth and rough *B. abortus* organisms at different pH's in the absence or presence of immunoglobulins.

## MATERIALS AND METHODS

**Bacterial cultures.** Organisms were grown at 37°C in a 5% $CO_2$-95% air atmosphere for 3 days on Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) solidified by the addition of 2% agar.

*B. abortus* strain 19 was subcultured from *B. abortus* vaccine vials (Colorado Serum Company, Denver, Colo.); *B. abortus* strain 45/20 was subcultured from lyophilized cells kindly provided by L. M. Jones (University of Wisconsin, Madison).

**Antigen preparations.** Antigen extracts were obtained from *B. abortus* strain 45/20 cells which had been grown in a fermentor at the National Animal Disease Center, U. S. Department of Agriculture, Ames, Iowa. Before antigen extraction, cells were treated with acetone as described before (13), washed twice in distilled water, and lyophilized. Antigenic preparations were extracted from the acetone-treated cells with hypertonic NaCl or from sonicates of these acetone-killed cells (2). The antigenic preparations contained antigens A1, A2, and A4 described previ-

ously (13), and antigen A5 to be described here. All four antigens are also detectable in extracts of *B. abortus* strain 19 cells (Schurig, unpublished data).

**Rabbit and pig antisera. (i) Anti-strain 45/20 extract antiserum.** Several New Zealand white rabbits were injected subcutaneously with the antigenic, hypertonic NaCl extract of *B. abortus* strain 45/20 incorporated in Freund complete adjuvant as described before (13). The sera from these rabbits were designated multispecific sera.

**(ii) Anti-strain 19 antiserum.** One rabbit (R-10) was immunized with autoclaved *B. abortus* strain 19 cells incorporated in Freund complete adjuvant. Two injections 14 days apart were administered subcutaneously, and the rabbit was bled 35 days after the first immunization.

**Monospecific pig antisera.** After immunoelectrophoresis (IE) of *B. abortus* strain 45/20 antigen extract (sonicate) and development of precipitation arcs with monospecific sera prepared in rabbits against antigens A1, A2, and A4 as described previously (13), the specific precipitin lines were cut out from the agarose slide and washed in 0.15 M saline containing $10^{-3}$ M ethylenediaminetetraacetic acid (EDTA) for 5 days with frequent changes of the saline-EDTA solution. After washing, the agarose pieces, containing 15 precipitin lines per animal to be injected, were minced, emulsified in an equal amount of Freund complete adjuvant, and injected intramuscularly into pigs as described previously for rabbits (13). Animals were bled out after the detection of specific antibody in IE. Monospecific sera to antigen A5 were prepared in a similar way by using serum from a bovine which developed three precipitation arcs, including one anodal reaction called "A5" in IE. Minced and washed A5 precipitin lines emulsified in Freund complete adjuvant were injected into rabbits to produce monospecific anti-A5 sera. The antibodies obtained in rabbits were again used to develop A5 lines after IE of the hypertonic NaCl antigenic extract to minimize contamination with precipitin lines corresponding to other antigens. These precipitin lines were treated as described above and injected into pigs for obtention of specific anti-A5 antibodies.

**Absorption of antisera.** Anti-*B. abortus* strain 19 (R-10) multispecific sera and monospecific anti-A1, -A2, -A4, and -A5 sera were absorbed with live *B. abortus* strains 19 and 45/20 cells and acetone-killed strain 45/20 cells. Live cells were washed twice in 0.01 M phosphate-buffered saline (PBS) (pH 7.2) before absorption. A 60-mg amount (wet weight) of live or killed cells was incubated with 0.5 ml of antisera at 37°C for 2 h under gentle mixing. Cells were sedimented by centrifugation, and the absorbed serum was tested for antibody activity in IE.

**Electron microscopy.** Live cells were washed twice in 0.01 M PBS (pH 7.2) or in acetate buffer (pH 4.3) and then suspended for 20 min at room temperature in antisera or purified pig immunoglobulin G (IgG) (Cappel Laboratories, Downingtown, Pa.) diluted to 1:5 in PBS or acetate buffer. The optimal dilution of antisera was established before its use in this study. In some instances, cells were incubated with normal goat serum for 20 min before the addition of the anti-brucella sera. Cells were then washed three times in PBS and thereafter suspended in a 1:2 dilution of a rabbit IgG fraction with anti-pig IgG activity conjugated to ferritin (Cappel Laboratories). This serum was tested in IE for the presence of serum fractions other than rabbit IgG, as well as for the presence of antibody activity against pig serum components other than IgG. The tests confirmed the claims of the manufacturer. After three washes with Sorenson buffer (pH 7.2), cells were fixed with 1.25% glutaraldehyde, postfixed with 2% osmium tetroxide, dehydrated in alcohol, embedded in Epon 812, sectioned, stained with uranyl acetate, and examined in an electron microscope as described elsewhere (17). Ruthenium red staining of cells not subjected to serum incubations was performed by the method of Patterson et al. (9).

**Microelectrophoresis of *B. abortus*.** Live *B. abortus* strains 19 and 45/20 organisms grown on solid media were washed twice in distilled water, heat-killed at 60°C for 15 min, and then lyophilized.

The electrophoretic mobilities or organisms at different pH's or of organisms preincubated in sera were measured with a cytopherometer (Carl Zeiss, Oberkochen, Federal Republic of Germany) fitted with platinum electrodes.

For pH mobility studies, sodium chloride/sodium acetate/sodium barbiturate/HCl buffers of ionic strength 0.05 were prepared as described by Gittens and James (4), except that the stock solution was diluted to ionic strength 0.05, and the pH was adjusted with HCl (ionic strength = 0.05). The conductivity of the various buffers was measured with a model 31 conductivity bridge (Yellow Springs Instrument Co. Inc., Yellow Springs, Ohio), fitted with a YSI 3403 conductivity cell. Cells were washed three times in buffer of the appropriate pH and suspended at 3 to 4 mg (dry weight) per ml. Measurements were done at 30.0 ± 0.2°C, and movement was normally timed over 48 μm in both directions (current reversed). Each mean mobility was calculated from at least 20 observations.

To detect binding of antibody by alteration of electrophoretic mobility, sera were diluted with either PBS (0.05 M, pH 7.0) or acetate-buffered (0.05 M, pH 4.0) saline, and 4 mg (dry weight) of cells was incubated with either 5 ml of diluted serum, purified pig IgG (Cappel Laboratories), or gamma-globulin-free fetal calf serum GIBCO Labortories, Grand Island, N.Y.) for 1 h at 37°C. The commercial products were tested for purity before use. The lyophilized cells were rehydrated before incubation by washing in the appropriate buffer. After incubation, cells were removed from suspension by centrifugation and suspended in pH 4.0 or 7.0 buffer (ionic strength = 0.05) as used in pH mobility studies. Brief sonication in a sonicator bath was used to disperse flocculent suspensions.

**Agglutination test.** The standard tube agglutination test (1) with *B. abortus* strain 1119-3 was performed.

## RESULTS

**Preparation of antisera.** Pig antisera to antigens A1, A2, A4, and A5 were usually obtained 20 days after the second immunization of the

animals. The sera were demonstrated to be specific for the antigens when tested in IE against hypertonic NaCl extracts or sonicates of *B. abortus* rough strain 45/20 (Fig. 1). Antigen A5 migrated toward the anode in a position similar to antigen A4 (13) (Fig. 1). The sera did not agglutinate the standard tube agglutination antigen or live *B. abortus* strain 19 cells washed in PBS. No activity against preparation of rough and smooth LPS (kindly provided by E. Moreno, Universidad Nacional de Heredia, Heredia, Costa Rica) could be detected in IE. Multispecific sera reacted against many antigenic components present in the *B. abortus* extract (Fig. 1) but did not agglutinate smooth *B. abortus* cells, nor did it react with rough or smooth LPS preparations in IE. The R-10 sera used in this study demonstrated a titer of 1:800 in the standard tube agglutination test. In IE tests, the R-10 serum reacted with smooth LPS preparations, did not react with rough LPS preparations, and revealed one small anodal precipitation arc with NaCl extracts of strain 45/20.

**Absorption of antisera.** A 60-mg amount (wet weight) of live and acetone-killed *B. abortus* strain 45/20 cells was able to absorb all of the A5 activity out of 0.5 ml of monospecific anti-A5 serum and multispecific sera (Fig. 2, Table 1). In addition to being able to remove the anti-A5 reactivity, acetone-killed cells were also effective in removing the anti-A1 activity from the respective monospecific serum and several other specificities from the multispecific sera. They were unable to absorb out the anti-A2 and -A4 activity from the respective monospecific antisera (Table 1). Live, washed strain 19 cells did not remove the antibodies against A1, A2, A4, or A5 but did remove the agglutinating activity of the R-10 serum.

**Electron microscopic observations.** Ferritin deposition could be observed along the outer membrane of approximately 80% of the *B. abortus* strain 45/20 cells treated with anti-A5 serum. Deposition of ferritin on the cell membrane was not uniform, with areas of the surface remaining untagged (Fig. 3). Approximately 65% of the *B. abortus* strain 45/20 cells treated with anti-A1, -A2, -A4, or R-10 sera, as well as preimmunization pig serum or purified pig IgG, remained virtually untagged. Nevertheless, several organisms within these preparations, particularly those incubated in purified pig IgG, did demonstrate ferritin tagging. This nonspecific tagging could not be eliminated when cells were incubated with the reagents buffered at pH 4.3 or other pH's. Tagging of organisms was reduced to 5% when the cells were incubated in normal goat serum before the incubation with the test sera, except with anti-A5 pig serum, in which instance approximately 60% of the organisms were still tagged after preincubation with normal goat serum. *B. abortus* strain 19 cells demonstrated ferritin deposition when incubated with R-10 serum, but not with any of the monospecific or multispecific antisera. The ferritin deposition on strain 19 cells was arranged in a patchwise fashion with several areas of the cell surface remaining untagged (Fig. 4). The IgG fraction of rabbit anti-pig IgG serum conjugated to ferritin was unable to tag *B. abortus* cells at the 1:2 dilution used.

Ruthenium red staining of organisms did not appear to enhance the outline of superficial structures on *B. abortus* cells.

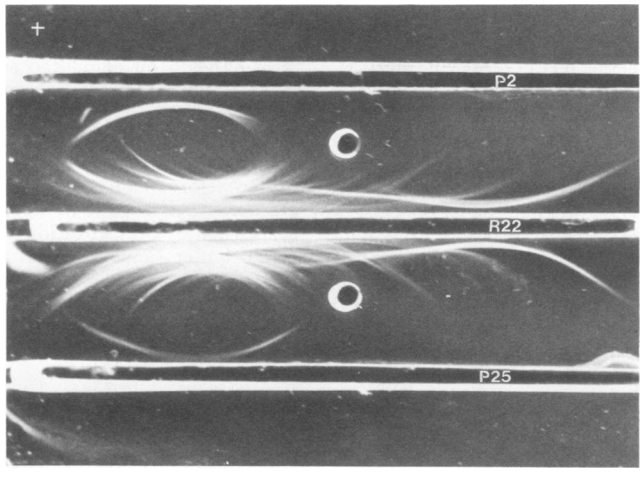


FIG. 1. *IE in agarose. Demonstration of monospecific anti-A5 serum (P25) and anti-A4 serum (P2). R22 is a multivalent anti-45/20 serum. Wells contain sonic extracts from strain 45/20. Anode is to the left.*

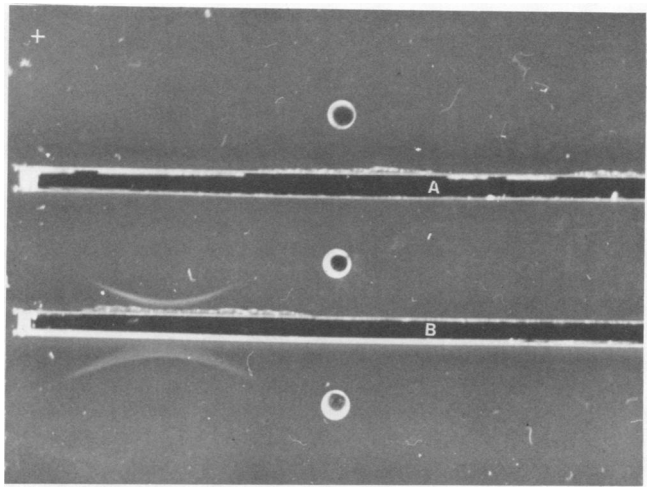


FIG. 2. *IE in agarose. After absorption of monospecific anti-A5 serum (A) with viable B. abortus strain 45/20 cells, the A5 precipitation line was removed. Well contains sonicate of 45/20 cells. Anode is to the left. B = Nonabsorbed anti-A5 serum.*

TABLE 1. *Absorption of monospecific antisera with live and acetone-killed B. abortus cells*

| Serum | Absorption with live *B. abortus*[a] | | Absorption with acetone-killed *B. abortus* 45/20 |
|---|---|---|---|
| | Strain 19 | Strain 45/20 | |
| Anti-A1 | − | − | + |
| Anti-A2 | − | − | − |
| Anti-A4 | − | − | − |
| Anti-A5 | − | + | + |

[a] −, No absorption of specific antibodies; +, absorption of specific antibodies.

**Electrophoretic mobility behavior of *B. abortus*.** The microelectrophoresis technique indirectly measures the net charge on the surface of particles. The greater the ionization of surface components, the greater the mobility value. Also, a negative mobility indicates that the surface was negatively charged and, therefore, cells migrated to the anode. The converse is also true. Figure 5 represents the effect of pH on the electrophoretic mobility of *B. abortus* strain 19 and 45/20 at an ionic strength of 0.05. The pH mobility curves produced by strains 19 and 45/20 are different and indicate a striking difference in the nature of the two surfaces. Strain 19 possessed a low degree of ionization of negatively charged groups, which was almost unaffected by pH. On the contrary, the mobility of strain 45/20 was drastically affected by pH, indicating the possession of highly ionized, positively charged groups below pH 4.2 and highly ionized, negatively charged groups above this pH value. The organisms were uncharged at pH 4.3.

**Detection of antibody binding to the cell surface of *B. abortus* by microelectrophoresis.** Microelectrophoresis, as previously stated, indirectly measures the net surface charge of a particle. Therefore, any binding of antibody to the surface will result in an alteration of electrophoretic mobility (6). It should, however, be noted that binding of antibody to the surface makes the mobility either increase or decrease, depending on the relative charge of the cell and the binding antibody.

Table 2 shows the electrophoretic mobilities of *B. abortus* strains 19 and 45/20 after incubation with sera. Mobilities for untreated cells were reproducible from experiment to experiment, with the exception of strain 45/20 at pH 4. The reason for more than normal variation in the mobility was probably attributable to slight changes in experimental conditions and difficulties in obtaining accurate measurements of slow-moving cells. However, cells gave a constant mobility for the duration of any particular experiment, thus any changes on incubation with serum indicate binding of antibody. There was no difference in the mobility of strain 19 when it was incubated with normal rabbit serum or pig anti-A5 serum at pH 7, but its mobility was significantly altered when it was incubated with the anti-strain 19 antiserum (R-10) at this pH, indicating that it binds to the surface. The mobility of strain 45/20 was changed significantly from that of the untreated cells when it was incubated with anti-A5 pig serum at pH 7, indicating binding of serum components to the surface. However, normal pig serum also bound to the surface of strain 45/20 at pH 7. This binding

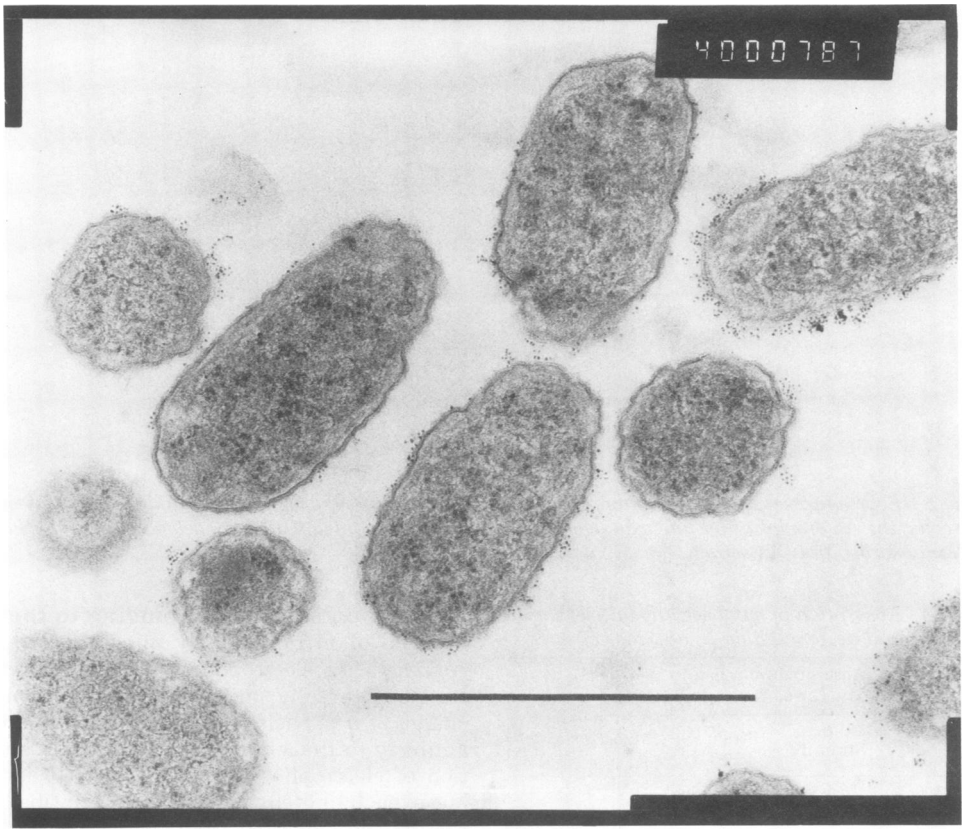

FIG. 3. *Ultrathin sections of B. abortus strain 45/20 cells treated with ferritin-conjugated anti-pig antibodies after incubation in monospecific pig anti-A5 serum. Scale = 1 μm.*

does not appear to be an electrostatic effect, since at pH 4.0, when cells possess a positive charge, binding is still apparent. The binding effect was also observed with other monospecific pig sera, purified pig IgG, and gamma-globulin-free FCS at pH 4.

## DISCUSSION

The present study indicates that antigens A1, A2, A4 (13), and A5 are not accessible to antisera on the surface of *B. abortus* smooth strain 19 cells. It does indicate that antigen A5 is present on the surface of at least a high proportion of *B. abortus* rough strain 45/20 organisms. The most convincing, although not definitive demonstration of the superficial location of antigen A5, is derived from the observation that live *B. abortus* strain 45/20 cells are able to absorb out anti-A5, but not anti-A1, -A2, and -A4 antibodies from the respective monospecific antisera. Electron microscopic observations revealed few broken organisms in the absorbing population, making it unlikely that organisms which lost their cell wall integrity during the washing and absorption process were responsible for the absorption of antigen A5. The ability of acetone-killed rough strain 45/20 cells of absorbing antibodies to several antigenic moieties indicates that the use of chemically treated *B. abortus* cells alter the results obtained with viable organisms and lead to false conclusions on superficiality of antigens.

The indirect ferritin-tagging experiments revealed that *B. abortus* rough strain 45/20 cells are able to bind purified pig IgG or IgG molecules present in normal, preimmunization pig and rabbit sera. This binding could not be prevented even when cells and sera were incubated and washed at a pH at which the organisms possessed a positive, instead of a negative, charge as demonstrated in the electrophoretic mobility experiments. This observation, in addition to the alterations observed in the electrophoretic mobility of rough strain 45/20 cells at close to an isoelectric point of pH 4.3 in the presence of pig IgG, virtually excludes a charge phenomenon as the causative agent of the immunoglobulin binding. The binding of gamma-

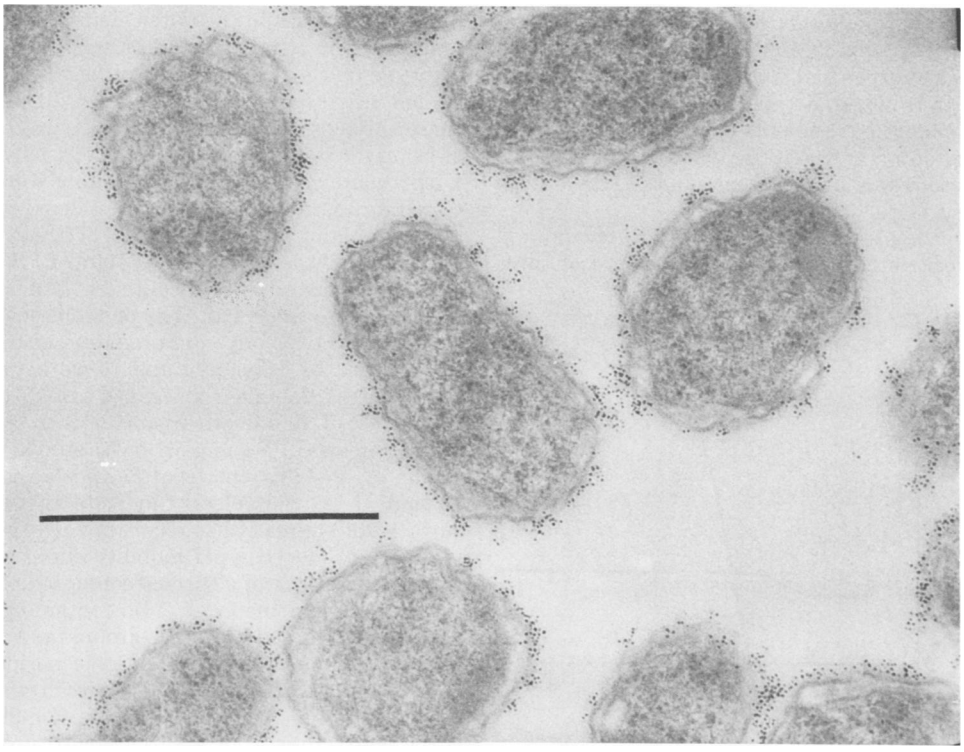

FIG. 4. *Ultrathin sections of B. abortus strain 19 cells treated with ferritin-conjugated anti-rabbit antibodies after incubation with R-10 serum. Notice untagged areas on cell surfaces. Scale = 1 μm.*

globulin-free fetal calf serum detected in microelectrophoresis indicates that immunoglobulins are not the only serum proteins able to bind to the rough strain; thus although it is possible to postulate the presence of an IgG receptor on this strain, we have not determined whether such a receptor is specific for the immunoglobulin.

The antigenically nonspecific binding of IgG molecules to the surface of *B. abortus* strain 45/20 organisms made the interpretation of antigen A5 visualization with ferritin-conjugated antibodies difficult. The ability to virtually block all of the nonspecific ferritin tagging of monospecific anti-A1, -A2, and A-4 sera, pig IgG, or preimmunization sera with normal goat serum indicates that components of the goat serum are blocking the nonspecific attachment sites for the pig IgG. The inability to block the tagging of anti-A5 serum would indicate that binding of anti-A5 antibodies was occurring in a specific way. Nevertheless, we were unable to label all strain 45/20 cells with anti-A5 serum. This could be due to cell-cycle-dependent superficial expression or even synthesis of antigen A5. Such a phenomenon has been reported to occur with the lambda receptor protein (5) and the 80,000-dalton protein of *Escherichia coli* (12).

In contrast to the *B. abortus* rough organisms, the smooth strain 19 organisms could only be tagged with the anti-strain 19 (R-10) serum. This specific-antibody-binding phenomenon was also reflected in the electrophoretic mobility test, in which only the R-10 serum was capable of changing the superficial charge characteristics of the smooth cells and, thus, change the electrophoretic mobility of the organism. Ferritin tagging was always in a patchwise fashion, regardless of the serum dilution used, indicating that some areas on the smooth cells do not react with the agglutinating antibodies present in R-10 serum. Diaz and co-workers (3) demonstrated the presence of the polysaccharide hapten poly-B on the surface of smooth Brucella organisms by absorption experiments. We were unable to demonstrate poly-B antibodies by radial immunodiffusion as described by Diaz et al. (2) in the R-10 serum, which could indicate that some of the untagged areas observed in this study may correspond to the location of the poly-B antigenic moiety or to some other antigenic determinants unrelated to the smooth LPS complex. On the other hand, the untagged areas may well correspond to antigenic moieties of the smooth LPS complex to which the R-10 serum does not

contain antibodies (8, 18). It is also possible that antigen-specific IgM molecules are bound to the untagged areas and were not detected with the ferritin-conjugated anti-IgG serum used, but preliminary experiments (G. G. Schurig, unpublished data), in which the IgG fraction of the R-10 serum was used, reveal similar gaps on the cell surfaces.

Utilizing electron microscopy, Raybould and co-workers (10) revealed the presence of three different antigens in the outer membrane envelope of smooth brucella organisms. This was achieved by the use of specific antisera (11) and pooled sera from brucella-infected cattle. One of these antigens, designated antigen X, was found to be on the surface of smooth brucella cells (10). Unfortunately, antigen X preparations were not tested for their content in 2-keto-3-deoxyoctonate, precluding any assessment of their LPS content, nor was the absence of anti-LPS antibodies demonstrated in the antisera used for the visualization studies (10). The possiblity of anti-smooth-LPS antibody contamination of the reagents used by Raybould and co-workers (10) may indicate that their antigen X visualized on the surface of the smooth organisms corresponds to the smooth LPS complex in our study.

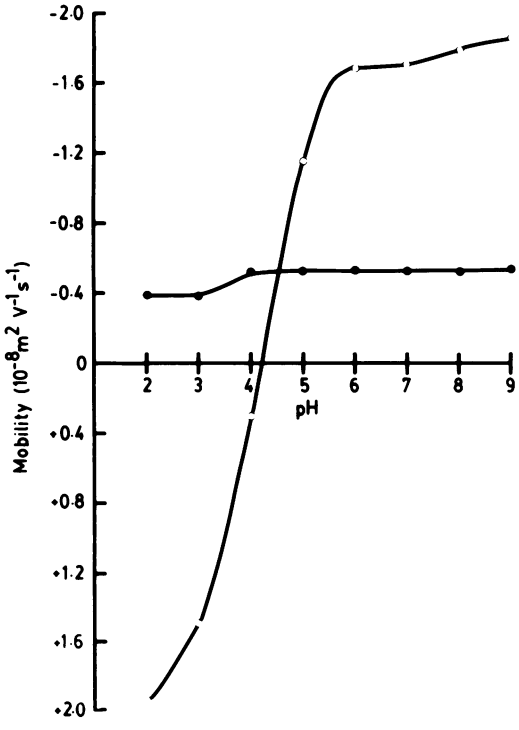


FIG. 5. *Mobility of B. abortus strain 19 (●) and 45/20 (○) cells in an electrical field at pH values ranging from 2 to 9.*

The pH mobility curves of *B. abortus* strains 19 and 45/20 cells clearly indicate that their surfaces differ greatly in composition. *B. abortus* strain 19 produced a pH mobility curve which was characteristic of a surface composed of polysaccharide-like material (14), containing few negatively charged carboxyl groups and most probably representing the polysaccharide O-chains of the smooth LPS molecule. Strain 45/20, on the other hand, exhibited a pH mobility curve characteristic of a surface with protein-like moieties (4, 14), in possessing ionizable positively charged (amine) groups, negatively charged (carboxyl) groups, and an isoelectric point of pH 4.2. This pH-dependent electrophoretic mobility suggests that as brucella organisms become rough, protein moieties in their outer membrane become exposed on the surface of the organism. A similar phenomenon was described by Smit and co-workers (15, 16) for smooth and rough *Salmonella typhimurium* organisms, using electron microscopy and ferritin-tagged antibodies.

It appears thus that, although brucella-in-

TABLE 2. *Electrophoretic mobility of B. abortus cells strains 19 and 45/20 incubated with sera or IgG at pH 4.0 or 7.0*

| Cells | Serum | Electrophoretic mobility[a] of cells after treatment with: | | | | pH[b] |
|---|---|---|---|---|---|---|
| | | Serum at dilutions of: | | | Buffer only | |
| | | 1:10 | 1:50 | 1:100 | | |
| Strain 19 | Normal rabbit | −0.5544 | −0.5507 | −0.5500 | −0.5592 | 7.0 |
| | R-10 | −0.6292 | −0.5674 | −0.5473 | −0.5592 | 7.0 |
| | Pig anti-A5 | −0.5311 | −0.5212 | −0.5178 | −0.5223 | 7.0 |
| Strain 45/20 | Normal pig | −0.8599 | −1.0934 | −1.2128 | −1.6778 | 7.0 |
| | Pig anti-A5 | −0.9225 | −1.0611 | −1.4053 | −1.6778 | 7.0 |
| | Normal pig | +0.9350 | +0.9291 | +0.9257 | +0.5170 | 4.0 |
| | Pig IgG | +1.4075 | +1.3838 | +1.3488 | +0.6545 | 4.0 |
| | GG-free FCS[c] | +1.4754 | +1.4237 | +1.4271 | +0.6545 | 4.0 |

[a] Mobility units are $10^{-8}$ $m^2$ $V^{-1}$ $s^{-1}$.
[b] pH of incubation and measurement.
[c] Gamma-globulin-free fetal calf serum.

fected or vaccinated cattle do produce antibodies to several antigenic components distinct from the smooth LPS complex of the microorganism (13), none of these antigens appears to be accessible to them on the surface of the smooth organisms, and only one of the studied antigens appears to be located on the surface of the rough cells.

## ACKNOWLEDGMENTS

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## LITERATURE CITED

1. **Alton, G. G., L. M. Jones, and D. E. Pietz.** 1975. Laboratory techniques in brucellosis. World Health Organization monograph series no. 55. World Health Organization, Geneva, Switzerland.
2. **Diaz, R., P. Garatea, L. M. Jones, and I. Moriyon.** 1979. Radial immunodiffusion test with a *Brucella* polysaccharide antigen for differentiating infected from vaccinated cattle. J. Clin. Microbiol. **10**:37–41.
3. **Diaz, R., L. M. Jones, D. Leong, and J. B. Wilson.** 1968. Surface antigens of smooth brucellae. J. Bacteriol. **96**:893–901.
4. **Gittens, G. J., and A. M. James.** 1963. Some physical investigations of the behavior of bacterial surfaces. IV. Chemical modification of surface components. Biochem. Biophys. Acta **66**:237–249.
5. **Gudas, L. J., R. James, and A. B. Pardee.** 1976. Evidence for the involvement of an outer membrane protein in DNA initiation. J. Biol. Chem. **251**:3470–3479.
6. **Hill, M. J., A. M. James, and W. R. Maxted.** 1964. Application of particle electrophoresis to the detection of antibody bound to cells of *Streptococcus pyrogenes*. Nature (London) **202**:187–188.
7. **Moreno, E., M. W. Pitt, L. M. Jones, G. G. Schurig, and D. T. Berman.** 1979. Purification and characterization of smooth and rough lipopolysaccharide from *Brucella abortus*. J. Bacteriol. **138**:316–369.
8. **Moreno, E., S. Speth, L. M. Jones, and D. T. Berman.** 1981. Immunochemical characterization of *Brucella* lipopolysaccharides and polysaccharides. Infect. Immun. **31**:214–222.
9. **Patterson, H., R. Irvin, and J. W. Costerton.** 1975. Ultrastructure and adhesion properties of *Ruminococcus albus*. J. Bacteriol. **122**:278–287.
10. **Raybould, T. J. G., J. E. Beesley, and S. Chantler.** 1981. Ultrastructural localization of characterized antigens of *Brucella abortus* and distribution among different biotypes. Infect. Immun. **32**:318–322.
11. **Raybould, T. J. G., and S. Chantler.** 1980. Serological differentiation between infected and vaccinated cattle by using purified soluble antigens from *Brucella abortus* in a hemagglutination system. Infect. Immun. **29**: 435–441.
12. **Ryter, A., H. Shuman, and M. Schwartz.** 1975. Integration of the receptor for bacteriophage lambda in the outer membrane of *Escherichia coli*: coupling with cell division. J. Bacteriol. **122**:295–301.
13. **Schurig, G. G., L. M. Jones, S. L. Speth, and D. T. Berman.** 1978. Antibody response to antigens distinct from smooth lipopolysaccharide complex in brucella infection. Infect. Immun. **21**:994–1002.
14. **Shaw, D. J.** 1969. Electrophoresis, p 44. Academic Press, Inc., London.
15. **Smit, J., and H. Nikaido.** 1978. Outer membrane of gram-negative bacteria. XVIII. Electron microscopic studies on porin insertion sites and growth of cell surface of *Salmonella typhimurium*. J. Bacteriol. **135**:687–702.
16. **Smit, J., K. Yoshiyuki, and H. Nikaido.** 1975. Outer membrane of *Salmonella typhimurium*: chemical analysis and freeze-fracture studies with lipopolysaccharide mutants. J. Bacteriol. **124**:942–958.
17. **Swaney, L. M., and S. S. Breese.** 1980. Ultrastructure of *Haemophilus equigenitalis*, causative agent of contagious equine metritis. Am. J. Vet. Res. **41**:127–132.
18. **Wilson, G., and A. Miles.** 1932. The serological differentiation of smooth strains of the *Brucella* group. Br. J. Exp. Pathol. **13**:1–13.