Putative Novel Surface-Exposed Streptococcus agalactiae Protein Frequently Expressed by the Group B Streptococcus from Zimbabwe

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Group B streptococci (GBS) express a variety of surface-exposed and strain-variable proteins which function as phenotypic markers and as antigens which are able to induce protective immunity in experimental settings. These proteins make it possible to define GBS serotypes, and GBS serotype-variable proteins are also important GBS markers. Among these proteins, the chimeric and immunologically cross-reacting alpha-like proteins are particularly important. Another protein, R3, which has been less well studied, occurred at a frequency of 21.5% in GBS from Zimbabwe and, notably, occurred in serotype V strains at a frequency of 75.9%. Working with rabbit antiserum raised against the R3 reference strain ATCC 49447 (strain 10/84; serotype V/R3) to detect the expression of the R3 protein, we recorded findings which suggested that strain 10/84 expressed a strain-variable protein antigen, in addition to R3. The antigen was detected by various enzyme-linked immunosorbent assay-based tests by using acid extract antigens or GBS whole-cell coats and by whole-cell-based Western blotting. We named the putative novel antigen the Z antigen. The Z antigen was a high-molecular-mass antigen that was susceptible to degradation by pepsin and trypsin but that was resistant to m-periodate oxidation and failed to show immunological cross-reactivity with any of a variety of other GBS protein antigens. The Z antigen was expressed by 33/121 (27.2%) of strains of a Zimbabwean GBS strain collection and by 64.2% and 72.4% of the type Ib and type V strains, respectively, and was occasionally expressed by GBS of other capsular serotypes. Thus, the putative novel GBS protein named Z showed distinct capsular antigen associations and presented as an important phenotypic marker in GBS from Zimbabwe. It may be an important antigen in GBS from larger areas of southern Africa. Its prevalence in GBS from Western countries is not known.

Streptococcus agalactiae (the group B streptococcus [GBS]) possesses genotypic and phenotypic markers which are important in epidemiological settings. Among the established phenotypic markers, the capsular polysaccharides (CPSs) Ia, Ib, and II through IX play prominent roles in the classification of GBS, in the pathogenesis of GBS disease, and as targets of protective antibodies (15). A variety of surface-anchored and strain-variable proteins are also important GBS markers.

These proteins make it possible to define GBS serotypes within each CPS type by using antibody-based or gene-based methods for subtyping (6, 11, 19, 22). These proteins include C8 and the alpha-like proteins (Alps) Cα, Alp1 (formerly epsilon), Alp2 and Alp3 (formerly probably R1), and Rib (another designation for the R4 protein) (28). The Alps are characterized, among other ways, by a repeat-containing region which has a causal relation to the ladder-like patterns formed by these proteins on sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and Western blotting. The Alps possess chimeric sequences, show variable immunological cross-reactivities, and induce increased resistance to GBS infections in experimental models (1, 13, 15, 18, 30). For this reason, these proteins can be considered vaccine candidates, either alone or as carrier proteins coupled to CPS (33, 34).

The frequency of occurrence of the surface-localized and strain-variable antigens may vary with the geographic location (10, 14, 19), meaning that it may be necessary to manufacture GBS vaccines with different formulations, depending on the area where the vaccine is to be used. The R3 protein of GBS seems to be an example of a GBS antigen with regional variation in the frequency of expression. This protein has been known for a long time (32) but has not yet been sequenced and, generally, has not attracted much interest among investigators, although some data indicate that it occurs in the Western World, but rarely (12). However, in two recent studies of GBS from Zimbabwe, it was found that R3 occurred at frequencies of 24% and 21.5%, respectively (19, 22), similar to the frequency of possession of the genes encoding Cα or Alp1 (19). Alp3, which encodes Alp3, was found in only 5% of one of the Zimbabwean strain collections and in only 6.9% of the type V strains (19). With GBS from Western countries, Alp3 (or alp3) has been detected in 60% to 90% of type V strains (1, 11, 13, 24).

In our work on carrier GBS strains from Zimbabwe, we have used a murine monoclonal anti-R3 antibody (R3 MAb) for the detection of R3 expression (12, 19, 22). When R3 MAb-negative strains were tested by using a polyclonal antibody (PAb) considered specific for the R3 protein, we recorded findings which led us to suspect the expression by certain isolates of a
strain-variable antigen which was different from R3 and from other of the well-defined, surface-exposed GBS antigens. In this report, we present the results of the experiments which supported this inference and present some characteristics of the putative novel GBS antigen, including its frequency of expression in a Zimbabwean GBS strain collection and its associations with CPSs.

MATERIALS AND METHODS

Bacterial strains. The reference and prototype strains used in this study have been described previously (12, 18) and included strains of all CPS serotypes except serotype IX (27) and isolates which expressed one or more of the following cross-reacting serotypic antigens: Cß, Cα, AlpI, Alp2, Alp3, and the molecule R3. Type III/R4, sequence type 17, strains were from the collection of clinical isolates of the Laboratory of Medical Microbiology, St. Olav’s Hospital, Trondheim, Norway. A total of 121 GBS strains from Zimbabwe were studied. Briefly, these isolates, which were recently described (19), were vaginal or rectal isolates collected during the period from 2003 to 2005 from pregnant Zimbabwean women. The isolates were collected, cultured, serogrouped, and serotyped as described previously (19). Todd-Hewitt broth or blood agar plates were used to culture the isolates at 37°C for 20 h or sometimes for 48 h.

Bacterial extracts. HCl extracts were prepared from some of the GBS strains. Bacteria cultured in broth were collected by centrifugation, washed with phosphate-buffered saline (PBS; pH 7.2), and suspended in 0.2 M HCl at 5 mg ml^-1 bacterial pellet. The suspension was heated at 50°C for 2 h, neutralized with NaOH, and spun at 10,000 × g for 10 min. After centrifugation, an equal volume of 10% (wt/vol) trichloroacetic acid was added to the supernatant, and the mixture was kept at 4°C for 20 h. The precipitate was collected by centrifugation, dissolved in 4 ml g^-1 extracted bacteria, dialyzed against PBS with 0.02% (wt/vol) NaNO3, and kept at 4°C or −20°C.

Antiserum. A murine R3 MAb and rabbit antisera were used. Both the MAb and the polyclonal antiserum (PAb) had been raised by immunization with whole cells of the R3 reference strain 10/84 (ATCC 49447; serotype V/R3 [12]). The MAb was of the immunoglobulin M (IgM) isotype (12). The rabbit antisera was absorbed with R3 whole cells to remove antibodies against common GBS antigens, thus generating polyclonal anti-R3 serum (the original R3 PAb). In a previous study, it was found that both the R3 MAb and the original R3 PAb recognize available prototype and reference strains, in accordance with the specificity for the R3 protein (12).

Absorption of antiserum. Antiserum was absorbed by the washed and pelleted bacteria, as described previously (2). Pellet volumes at least two times the volume of undiluted antiserum were used for absorption.

Coating antigens. HCl extracts of GBS or bacterial whole cells were used for antigens in an enzyme-linked immunosorbent assay (ELISA). A suspension of ~10^9 bacteria ml^-1 was made in PBS-NaNO3. The bacteria had been cultured for 48 h, and the suspension had been frozen and thawed at least one time. Optimal coating concentrations were determined by checkerboard titrations and were usually 1.8 for the bacterial extracts and 1.4 to 1.8 for the bacterial suspensions.

ELISA. ELISA was performed as described previously (21) by using PBS with 0.05% (vol/vol) Tween 20 as the diluent and for the washings, reagent volumes of 50 µl in duplicate tests, alkaline phosphatase-conjugated antibodies to rabbit IgG (Sigma-Aldrich, St. Louis, MO) or murine IgM (Sigma-Aldrich), and p-nitrophenyl phosphate for the substrate. Twofold dilutions or appropriate single dilutions of antiserum were tested. Recordings of the optical density at 405 nm (OD405) were converted to an ELISA ratio, calculated as the mean for the recordings divided by the background recording, which ranged from 0.100 to 0.200. The ELISA titer was defined as the reciprocal of the highest serum dilution with an ELISA ratio of at least 2.0. PAB (1:2,000) or MAb (1:1,000) was used in a whole-cell-based ELISA to test the GBS strains for antigen expression. An ELISA ratio of ≥3.0 was chosen as the criterion for antigen expression. ELISA inhibition was performed by preparing dilutions of the bacterial extracts to which equal volumes of antiserum (1:1,000) were added, and the mixture was incubated at 20°C for 1 h. The mixture was tested by indirect ELISA, as described above. The antibody-neutralizing activity of the inhibitor was calculated as the percent reduction of the OD signaling of the positive control, which contained plain buffer as the inhibitor.

Western blotting. Materials solubilized from whole cells of GBS by hot SDS treatment were tested and targeted by rabbit antiserum, as described previously (23), except that NuPAGE Novex bis-Tris gels (Invitrogen) were used.

RESULTS

A putative novel GBS antigen. In previous studies of the distribution of serotypes among GBS from Zimbabwe, a murine MAb against the GBS protein R3 (R3 MAb) was used for whole-cell-based dot blotting (19, 22). Later, we reexamined some of the isolates by using the original anti-R3 PAb. Both the MAb and the PAB had been raised by immunization with whole cells of R3 reference strain 10/84 (ATCC 49447; V/R3 [12]), after which the rabbit antiserum had been exhaustively cross-absorbed by a serotype V/Alp3 GBS isolate. We concluded that the original anti-R3 PAb was specific for the R3 protein (12). The PAb and MAb showed concordant results by dot blotting, except that an occasional GBS strain which was negative with the R3 MAb showed a positive test result with the original R3 PAb. This observation triggered further experiments to test if the original R3 PAb contained antibodies both against R3 and against an additional antigen.

Table 1 shows the ELISA titers obtained with the original R3 PAb tested against HCl extracts from homologous R3 reference strain 10/84 (V/R3), reference strain 9828 (non-typeable [NT]/Alp4, R3), and Zimbabwean strains CMF930 (Ib/Co) and CMV223T (Ib/Co, Cб) before and after cross-absorption of the original R3 PAb by each of three of the isolates. The original R3 PAb recognized an HCl extract antigen(s) from all four isolates, but on absorption, only bacteria of homologous strain 10/84 depleted the antiserum of antibodies which targeted antigens of all of the extracts. These results accorded with the supposition that the original R3 PAb contained antibodies against more than one antigen present in the 10/84 extract. Preimmune serum showed titers of antibody against these antigens of <500. Table 2 shows the results of exhaustive absorption of the antibodies which recognized the
antigens used in antigen-antibody combinations 1 and 2, respectively, defined in footnote a of Table 2, and with the antisera diluted 1:2,000. Strains 10/84 and 9828 shared the antigen recognized by antibodies active in antigen-antibody combination 1. This antigen most likely was the R3 protein, in accordance with the known expression of R3 by both of these reference strains. We have named the corresponding antibody, i.e., the antigen-antibody combination 1 antibody, the R3 PAb. The antigen recognized by the antibodies active in antigen-antibody combination 2 was shared by strains 10/84, CMFR30, and GMFV223T; it was immunologically distinct from R3, and was not expressed by strain 9828. We have named this antigen, which has probably been undetected up to now, the Z antigen (for Zimbabwe) for this presentation, and we have named the corresponding antibody Z PAb. The results of whole-cell ELISA testing, shown in Table 3, confirmed the findings and interpretations described above, in that the R3 PAb recognized strains 10/84 and 9828, in accordance with R3 expression by these strains, while Z PAb recognized 10/84, CMFR30, and GMFV223T, in accordance with the expression of the Z antigen by these isolates. ELISA inhibition with HCl extracts for antigen coating and antibody inhibition confirmed the inferences mentioned above and provide evidence of identity with respect to the immunological specificity of the Z or R3 antigen originating from different isolates (Fig. 1). The results shown in Table 1 and Table 2, which were obtained with HCl extracts for coating, were the same when whole-cell coats were used. Tables 1, 2, and 3 and Fig. 1 show representative results of single experiments, which were confirmed by experiments that were repeated. It is important to note that the R3 MAb targeted the isolates (and HCl extract coats; data not shown), in accordance with specificity of this antibody for the R3 protein (Table 3), as was originally concluded for this antibody (12).

**Specificity testing.** The Z and R3 PAbs were tested by whole-cell ELISA with a variety of reference and prototype strains. Negative test results supported the notion that none of the antisera cross-reacted immunologically with Cβ, Cα, Alp1 (epsion), Alp2, Alp3, R4 (Rib), or the γ or β proteins, which are expressed by strain A909 (25). Negative test results for four clinical serotype III/R4, sequence type 17 strains make cross-reactivity with the repeat protein Srr-2 unlikely (26), and negative test results with strain ATCC 12403 (strain NEM316) makes cross-reactivity with the Srr-1 protein unlikely (26).

**Additional features of Z and R3.** Coating for ELISA with pepsin-digested 10/84 HCl extract resulted in a reduction of the OD<sub>abs</sub> of more than 95% with both the R3 and the Z PAbs. Coating with the trypsin-digested preparation resulted in reductions of the OD of 88% and 87% for the R3 and Z PAb, respectively. This is consistent with the extensive degradation of both R3 and Z by both pepsin and trypsin digestion but, with the latter enzyme, with breakdown products which still were able to bind some of the antibodies, as was shown for R3 in a previous study (12). Sodium m-periodate oxidation did not affect the antibody binding capacity of Z or R3. These findings strongly support the notion that both R3 and Z are proteins. Heating at 100°C for 10 min did not affect the antibody-binding ability of Z or R3.

Figure 2 shows the result of double-diffusion testing of HCl extracts against the original anti-R3 PAb, which contained PAbs against both the Z and the R3 antigen. A single precipitation line was formed by both of the R3-expressing strains, strains 10/84 and 9828, and there was a reaction of identity between the lines. Similar lines that also had a reaction of identity were formed when the testing was done with the R3-specific PAB (data not shown), consistent with generation of the lines by the R3 protein and anti-R3 antibodies and in agreement with the ELISA-based results. The strain 10/84 extract, which also contained the Z antigen, generated only the
R3 line; and HCl extracts from isolates CMFR30 and GMFV223T, both of which produced the Z antigen but not the R3 antigen, did not produce visible lines with any of the antisera. Variations in antigen and antibody concentrations or the addition of urea to the antigen solution until the final urea concentration was 9 M resulted in no formation of visible precipitation lines with the CMFR30 and CMFV223T extracts and antisera. It is a possibility that the Z antigen migrated very slowly in the agarose gel due to the formation of molecular aggregates. On chromatography with Sephacryl S-300 HR (Fig. 3A), a column material with a fractionation range of 10 kDa to 1,500 kDa for globular proteins, the strain 10/84 Z antigen displayed considerable heterogeneity and appeared with two molecular variants with respect to the molecular masses. The first peak had a molecular mass close to that of human IgM (900 kDa). R3 appeared to have less heterogeneity and had peak activity corresponding to about 500 kDa. Both the Z and the R3 antigens showed heterogeneity with respect to charge, and some difference between the proteins was found by ion-
mass of with an upper band positioned corresponding to a molecular antigens from both strain 10/84 and strain CMFR30 presented proteins presented with multiple bands on immunoblotting. The Z CMFR30 expresses Z but not R3 (Fig. 4A and B). Both pro-

The 10/84 and 9828 R3 proteins also showed patterns with multiple bands on Western blotting and an upper band corre-

exchange chromatography (Fig. 3B). The Z antigen from strain CMFR30 was eluted from these columns, similar to the case for the strain 10/84 Z antigen (data not shown). These results suggest difficulties in achieving the purification of Z and R3 by conventional chromatographic methods.

SDS lysates from strains 10/84, 9828, and CMFR30 and from other Z antigen-positive and -negative Zimbabwean strains were tested by Western blotting. The results confirmed that Z and R3 are two distinct proteins and that strain 10/84 expresses both Z and R3, strain 9828 expresses R3 but not Z, and strain CMFR30 expresses Z but not R3 (Fig. 4A and B). Both proteins presented with multiple bands on immunoblotting. The Z antigens from both strain 10/84 and strain CMFR30 presented with an upper band positioned corresponding to a molecular mass of >300 kDa and with stained bands of decreasing molecular mass down to ~150 kDa, with the most distinct banding pattern being detected for the CMFR30 Z antigen. With the 9828 lysate, anti-Z generated a band in the ~123-kDa position, and this was observed in several experiments but was not seen with other Z-antigen-negative isolates. We have no explanation for this line, but a nonimmunological molecular interaction or the recognition of a released cross-reacting intracellular 9828 antigen are possible explanations. The Z PAb showed no binding by ELISA with 9828 whole cells or HCl extract coats. The 10/84 and 9828 R3 proteins also showed patterns with multiple bands on Western blotting and an upper band corresponding to a molecular mass of ~150 kDa. The molecular masses of the Z antigens expressed by different isolates varied, as seen in Fig. 4C, consistent with strain-to-strain variations in molecular structure. The banding patterns seen on Western blotting resemble those seen with alpha-like GBS proteins (15).

Testing by indirect immunofluorescence showed that the R3 PAb resulted in distinct, linear, peripheral signaling with GBS which was shown by whole-cell ELISA to express R3 and no signaling with R3-negative bacteria. Anti-Z also resulted in distinct peripheral staining of Z-positive bacteria but with distinct stained dots at one or more sites at the bacterial cell surfaces and no signaling with Z-negative bacteria (data not shown). These results support other findings which suggest that both Z and R3 are surface-exposed proteins.

Distribution of Z in carrier GBS strains from Zimbabwe. Table 4 shows the results of a whole-cell-based ELISA of 121 carrier GBS strains from Zimbabwe obtained when the bacteria were probed with the Z PAb diluted 1:2,000. The CPS types of the bacteria had been determined by PCR (19). Expression of the Z antigen was detected in 33/121 (27.2%) of the isolates, 5.3% of the CPS type Ia strains, 64.2% of the type Ib strains, 10% of the type Ia strains, 72.4% of the type V strains, and one of two NT strains. Thus, Z antigen was expressed most often by CPS type Ib and V bacteria, meaning that Z-antigen expression showed a distinct CPS antigen association, although this association was not absolute (Table 4.). These data support the notion that Z antigen is a predominant strain-variable protein in the Zimbabwean GBS strains tested and has distinct CPS type associations.

DISCUSSION

In previous studies, GBS from Zimbabwe were tested for the expression of the R3 protein by using R3 MAb (19, 22). When the results were confirmed with R3 PAb, an occasional isolate showed discrepant results with the two antibodies. Through ELISA-based testing and Western blotting, evidence was provided that the discrepancy was due to antibodies in the R3 PAb which recognized another antigen, named the Z antigen by us, in addition to the antibodies which recognized R3. The Z antigen was expressed by a share of the Zimbabwean GBS strains and by one of our GBS reference strains, serotype V/R3 strain 10/84 (ATCC 49447). The Z antigen has to our knowledge not been described previously, although it may have been observed in SDS-PAGE and Western blot patterns (1). While strain 10/84 expressed both Z and R3, isolates which expressed only one of the two antigens were identified, which made it easy to prepare PAb considered R3 or Z antigen specific. The Z antigen was surface exposed, was susceptible to pepsin and

![Western blot of SDS lysates of whole cells of GBS strains.](image)
trypsin digestion, and resisted m-periodate oxidation, consistent with the protein nature of the antigen. Its susceptibility to trypsin digestion was similar to that of R3 (12), and this makes a distinction between the Z and R3 antigens on the one hand and the trypsin-resistant alpha-like proteins of GBS on the other (3, 8).

The R3 and Z antigens showed no immunological cross-reactivity; and the Z PAb failed to cross-react with Cb, Ca, Alp1, Alp2, Alp3, and R4 (Rib), as was also previously shown for anti-R3 antibodies (12). Anti-Z also failed to recognize a number of other GBS antigens. Cross-reactivity with still other surface-exposed GBS protein antigens, such as the C5a peptidase (5), the laminin-binding protein (29) or fibronectin-binding protein (25), the Sip protein (4), and additional antigens, has not been examined by antigen-specific tests. However, these antigens are produced by many if not all GBS strains (15), while Z antigen was expressed by a limited share of the 121 Zimbabwean isolates examined. The identity of Z antigen with the recently described R5 protein can be excluded, since R5 was detected in strain 9828 (7), which was negative for Z antigen. The failure of most of our reference and prototype strains to express the Z antigen (the exception was strain 10/84, which expressed both Z and R3) and the use in our laboratory of the R3 MAb to identify R3 by GBS serotyping explain why we did not suspect the existence of Z earlier. It is important to note that the targeting of the R3 protein by the R3 MAb described previously (12) was confirmed in the present study (Table 3).

The Z antigen failed to generate precipitate with antibodies in agarose gels, contrary to the case for R3. We have no obvious explanation for this. The formation of molecular aggregates with feeble migration in the gels could be an explanation. However, on Sephacryl S-300 HR fractionation, both Z and R3 were eluted and corresponded to high-molecular-weight proteins; a part of the 10/84 Z antigen had a molecular mass close to 1,000 kDa, which suggests aggregate formation. Both Z and R3 showed heterogeneity with respect to size and also with respect to charge, as found by ion-exchange chromatography. Heterogeneity may have resulted from or been augmented by the harshness of the HCl extraction method used by us. Immunoblotting of SDS lysates of whole cells of GBS revealed that both Z and R3 formed patterns with multiple bands over a wide range of molecular mass positions and with the upper bands having very high molecular masses, notably for the Z antigen. For R3, these findings, which were recorded when the R3 PAb was used as the probe, mimicked those found when the R3 MAb was used as the probe (12, 19). The multiplicity of molecular species of these proteins may have contributed to the heterogeneity recorded by chromatographic procedures (Fig. 3). It remains unknown whether the molecular diversity (i) resulted from events that occurred during protein synthesis, (ii) was caused by degradation by bacterium-produced enzymes, (iii) was caused by breaking of labile bonds during sample preparation (31), or (iv) was related to molecular structures with multiple large repeats, as is the case for the alpha-like GBS proteins (15, 16, 20). Our impression is that R3 and Z resemble each other in their physicochemical characteristics and, possibly, may belong to a separate GBS protein family. However, the lack of immunological cross-reactivity between them, like the cross-reactivity which exists between different alpha-like proteins (17, 18), indicates considerable structural dissimilarity. Sequencing of the encoding genes is required to establish if there is a structural relationship between Z and R3.

In 1999, a mutanolysin extract from strain 10/84 was described (1). The extract contained two proteins which migrated in SDS-polyacrylamide gels as ~110-kDa and ~100-kDa components and appeared together during chromatographic purification procedures. They were considered two variants of the same protein species which was called Fbs (type five, group B, surface protein). Fbs was susceptible to trypsin and pepsin digestion and was expressed by 31% of the serotype V GBS. The similarity of the findings recorded for Fbs and those recorded in the present study for the 10/84 Z and R3 antigens makes it possible that one of the Fbs protein variants was the R3 protein and the other was the Z antigen, although the Western blot patterns were different. Different extraction methods and other methodological differences may have contributed to this. It is important to note that on immunization with Fbs, enhanced resistance to GBS infection was induced in animals (1). However, it is not known which of the two Fbs polypeptides induced the enhanced resistance, i.e., whether Z or R3 induced the protection, if our suggestion is pursued.

Z-antigen expression was detected in 33/121 (27.2%) of the Zimbabwean GBS strains, according to the whole-cell-based ELISA results for many isolates confirmed to produce the Z antigen by Western blotting. A comparatively strict criterion for a positive test result was used by us, and we cannot exclude the low-level expression of the Z antigen by one or more isolates found to be Z antigen negative by us. Irrespective of this, the frequency of expression of the Z antigen was high and in the strain collection evaluated in the present study was similar to the frequency of expression of the R3 protein and to the frequency of possession of alp1 and bea (19), the genes encoding Alp1 and Ca, respectively; but the frequency of Z-antigen expression was lower than the frequency of possession of rib (19), the gene encoding the R4 (Rib) protein (31). Surface-anchored and strain-variable GBS protein antigens show associations with CPSs, such as the CPS type Ia/Alp1, type Ib/Ca, C8, type III/R4 (Rib), and type V/Alp3 associations (9, 11, 22, 24). The Z protein showed a strong preference for strains of CPS types Ib and V. Alp3 is known to be the predominating surface-anchored and strain-variable protein in CPS type V strains (9, 11, 24), but this was not the case with the type V GBS strains from Zimbabwe. Only 6.8% of the Zimbabwean type V strains possessed alp3, which encodes Alp3, while the R3 protein occurred in 75.9% of the type V isolates (19). The high frequency of expression of the Z antigen by the type V GBS strains found in this study adds to the previously found high frequency of the type V-R3 combination as characteristics which seemingly distinguish Zimbabwean type V GBS strains from those which predominate in other geographical areas, but with one important element of uncertainty, namely, that the frequency of Z-antigen expression by GBS strains from other areas is not yet known. Information on R3 expression is also sparse (8, 12). More data along these lines are needed, but for the time being, it seems that serotype V GBS strains from Zimbabwe and possibly from larger areas of southern Africa frequently possess serotype V markers which differ markedly from those of type V GBS strains from other
geographical areas. Numerous aspects of the Z and R3 proteins need elucidation, including their molecular structure and function, in particular, their immunobiological roles in GBS colonization and clinical infection and their potential as vaccine candidates. The GBS strains tested by us were carrier isolates. Testing of invasive strains from Zimbabwe could provide valuable information on the role of Z, R3, and other GBS markers in invasive GBS disease in areas of southern Africa. The Z antigen may influence GBS serotype designations in the future; for instance, the serotype designations of strains 10/84 (V/R3) and CMFR30 (Ib/Cß) may change to the serotype designations (V/R3, Z) and (Ib/Cß, Z), respectively.

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