Specificity and Prevalence of Natural Bovine Anti-Alpha Galactosyl (Galα1-6Glc or Galα1-6Gal) Antibodies

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Immunity against the carbohydrate components of microorganisms mediated by antibodies is an important part of host defenses. Humans and closely related primates, but not other mammals, possess natural anti-Galα1-3Gal antibodies which also, although less avidly, react with melibiose (Galα1-6Glc). Using an enzyme-linked immunosorbent assay (ELISA) with melibiose-bovine serum albumin as an antigen, we analyzed bovine anti-alpha galactosyl antibodies with respect to specificity and distribution in individual animals. Inhibition assays showed that melibiose was the strongest inhibitor, followed equally by stachyose (Galα1-6Glcα1-2Fru) and raffinose (Galα1-6Glcα1-2Fru) and then by Galβ1-6Gal, Gal, and Galα1-2Gal. Others, including Galα1-3Gal and Galα1-4Gal, only exhibited minor inhibition. Thus, these bovine anti-alpha galactosyl antibodies appeared to preferentially react with Galα1-6Glc or Galα1-6Gal. The distinction of this specificity from that (Galα1-3Gal) of human antibodies was further demonstrated by the poor reaction of bovine serum to the Galα1-3Gal antigen in comparison to human serum. All 27 healthy bovine serum samples of the three age groups (newborn, calf, and adult) tested contained such antibodies with titers increasing with age. The antibodies purified by affinity chromatography using a melibiose-agarose column were mainly of the immunoglobulin G (IgG) isotype with a concentration of >23 μg/ml in most samples. IgG1 was found to be the primary antimelibiose IgG isotype in all age groups by isotype-specific ELISA, but a significant increase in IgG2, an isotype more related to innate immunity, was observed in calves and adults, compared to newborns. The purified antibodies reacted with the type II bovine strain of Streptococcus agalactiae, a common pathogen of bovine mastitis. Thus, these anti-Galα1-6Glc or Galα1-6Gal antibodies in cattle might be involved in defense against microbes bearing this or the related epitopes.

Humans and closely related mammals possess natural anti-alpha galactosyl (Galα1-3Gal) antibodies (22). It is suggested that these human antibodies are developed in response to normal bacterial flora in the intestine (8) in a way similar to the blood group antigen-specific antibodies (34). The anti-alpha galactosyl (Galα1-3Gal) antibodies are found in humans, apes, and Old World monkeys but not in New World monkeys and other mammals (7, 9). This is consistent with the fact that humans, apes, and Old World monkeys lack a functional alpha-1-3 galactosyltransferase (14, 19) and therefore do not possess the Galα1-3Gal structure on their tissues. The opposite is true in other mammalian species, including cattle (13, 18), and the Galα1-3Gal structure is therefore a self antigen in these species.

The human anti-Galα1-3Gal antibodies have an unusually high titer, accounting for as much as 1% of total serum immunoglobulin G (IgG) (20 to 100 μg/ml) (5). They have been suggested to play an important role in innate immunity. They are responsible for inactivation of retrovirus and other enveloped viruses of animal origin by human serum (21, 28, 32) and rejection of pig-to-human xenografts (23). They may also be involved in preventing microbial and parasite infections (1, 30).

Although the human anti-alpha galactosyl antibodies react most avidly with the Galα1-3Gal structure, they also react strongly with melibiose (Galα1-6Glc) (6, 35). As a result, melibiose has been used as a ligand for the affinity purification of these human antibodies. Recently, antimelibiose antibodies have been detected in cattle and chickens (26, 27), suggesting that there are similar anti-alpha galactosyl antibodies in animals. However, their specificity and distribution have not been extensively studied and no comparison has been made with the Galα1-3Gal epitope. Here we report the characterization of the specificity and distribution of the bovine antimelibiose antibodies and their potential involvement in host defense against microorganisms.

MATERIALS AND METHODS

Bacteria. Streptococcus agalactiae types II (ATCC 27541), IV (ATCC 49446), and V (ATCC 700048) were obtained from the American Type Culture Collection. Type II is a bovine strain isolated from a cow with mastitis, whereas types IV and V are strains from humans. A clinical isolate of S. agalactiae obtained from the milk of a cow suspected of having mastitis and designated 5247 was provided by the Texas Veterinary Medical Diagnostic Laboratory (College Station). Escherichia coli (ATCC 25922 and ATCC 35215), Staphylococcus aureus (ATCC 29213 and ATCC 25923), and Pseudomonas aeruginosa (ATCC 27853) were also used. All bacteria were maintained on blood agar. They were cultured in tryptose broth for 24 h at 37°C before used in dot blot analysis.

Serum samples. One fetal, three newborn (1 to 10 days old), and five calf (6 months old) bovine pooled serum samples were obtained from Gibco-BRL (Grand Island, N.Y.). Nineteen adult sera were obtained from cows at ages of 1 to >10 years in the Veterinary Medicine Park, Texas A&M University. All adult samples were collected in October 1996. An older sample collected in May of 1992 from one cow was also used and designated 16-1. The new sample from this same cow was designated 16-2. All serum samples were stored at −20°C. A pooled adult sample was made by mixing equal volumes of individual adult samples, excluding samples 1, 6, 12, and 16-1 because of the limited amounts available. Two pooled adult human serum samples were used, one (I) from Sigma Chemical Co. (St. Louis, Mo.) and another (II) from Scantibodies Lab, Inc. (Santee, Calif.).

ELISA. An indirect enzyme-linked immunosorbent assay (ELISA) was used to measure serum antimelibiose antibodies. The assay was performed in a reagent excess manner; both the antigen and secondary antibody were used in excess amounts as determined by titration assays. Plates (NUNC, Inc., Naperville, Ill.) were coated overnight at 4°C with melibiose (Galα1-6Glc)-bovine serum albumin

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Plates were washed three times with wash buffer (phosphate-buffered saline [PBS]) containing 0.02% NaN3 and then blocked for 1 h with wash buffer containing 3% BSA (gamma globulin free; Sigma Chemical Co.), 0.02% Tween 20, and 0.02% NaN3 in PBS. PBS. Serum samples diluted in the same buffer were added to the plates (100 μl/well) and incubated at room temperature for 2 h. Following washing as described above, affinity-purified alkaline phosphatase-conjugated anti-human IgG whole molecule (Sigma Chemical Co.), anti-bovine IgG whole molecule (Sigma Chemical Co.), or alkaline phosphatase-conjugated anti-bovine IgM heavy (μ) chain antibodies (KPL, Gaithersburg, Md.) at a 1:1,000 dilution were added (100 μl/well) and incubated at room temperature for 1 h. After being washed, each well on the plate received 100 μl of the substrate p-nitrophenylphosphate prepared in accordance with the instructions from the manufacturer (Pierce, Rockford, Ill.). The reaction was stopped after a 10-min incubation by addition of 50 μl of 2 M NaOH. Optical density (OD) at 405 nm was determined using a Dynatech microplate reader (MR600).

For inhibition assays, sugars were first serially diluted twofold in the dilution buffer. Each dilution was then mixed with an equal volume of a diluted reference serum sample (calf sample 2). The mixture was then kept at room temperature for 1 h before being added to the plates. The remaining steps were the same as those described above. The inhibition efficiencies of various sugars were compared by determining the lowest concentrations that gave a 50% reduction in OD. The monosaccharides, disaccharides, and oligosaccharides used were obtained from Sigma Chemical Co., except for Galα1-3Gal, which was obtained from V-LAB.

Statistical analysis of ELISA data. A pooled calf serum sample (no. 2) was used as a reference for determining titers of individual samples. The titration endpoint was arbitrarily chosen as the highest serum dilution that gave an OD twofold higher than that of the background (fetal bovine serum). The endpoint was assigned as 1 titer unit/100 μl. This gave calf sample 2 a unit range of 1 (1:40) to 64 (1:1,024). The OD values were plotted against the logarithm of the titer units. A linear fit was made, and the log unit value of each sample was obtained by extrapolating against the regression line. The titer units per 0.1 ml of serum were calculated by an anti-log transformation, followed by multiplication by the dilution factor. The endpoint of calf serum 2 was found to be 1:1,000. The end point units per 0.1 ml of 2 M NaOH. Optical density (OD) at 405 nm was determined using a Dynatech microplate reader (MR600).

Measurement of anti-alpha galactosyl IgA, IgG1, and IgG2. Anti-alpha galactosyl IgA, IgG1, and IgG2 were measured by an indirect ELISA using affinity-purified anti-bovine IgA, IgG1, and IgG2 antibodies conjugated to horseradish peroxidase (Bethyl Laboratories, Montgomery, Tex.). The ELISA was performed as described above in a reagent excess manner. The conjugates or secondary antibodies were used at a 1:3,000 dilution. All serum samples were diluted 1:40. Tetramethylbenzidine (Pierce) was used as the substrate. The reaction was stopped after a 30-min incubation by addition of 100 μl of 2 M H2SO4. OD at 450 nm was determined using a Dynatech microplate reader (MR600).

Anti-alpha galactosyl IgA, IgG1, and IgG2. A 10-ml column of melibiose-agarose (Sigma Chemical Co.) was used for isolation of the bovine antimelibiose antibodies. The column was first washed with 100 ml of TN buffer (20 mM Tris, 150 mM NaCl, pH 7.4) containing 0.02% NaN3. Bovine serum (20 ml) mixed with an equal volume of TN buffer was loaded onto the column at a rate of 35 ml/h. The column was then washed with 200 ml of the buffer, and bound proteins were eluted with 200 mM melibiose in the same buffer. One-milliliter fractions were collected. Those fractions containing detectable proteins were pooled, dialyzed against TN buffer, and concentrated with polyvinylpyrrolidone (molecular weight, 360,000; Sigma Chemical Co.). Protein concentrations were determined by the bicinchoninic acid protein assay (Pierce).

Gel electrophoresis and immunoblotting. Electrophoretic analysis of isolated proteins was carried out under denaturing conditions as described previously (17). Proteins were detected by Coomassie blue staining.

For immunoblot analysis, proteins were blotted from the gel onto Immobilon P polyvinylidene difluoride membranes. Blotted membranes were first blocked at room temperature in TN buffer (25 mM Tris, 150 mM NaCl, pH 7.4) containing 3% BLOTTO and 0.05% Tween 20. The remaining steps with affinity-purified, alkaline phosphatase-conjugated antibody used to coat each well but rather performed under the same conditions to provide a relative comparison. It was found that the amount of bacteria attached to the membrane was consistent.

One-half microliters of the killed-bacterial preparations were spotted onto dry cellulose membranes and air dried. Coomassie blue staining was performed to ascertain that the amount of bacteria attached to the membrane was consistent. The membranes were blocked with 3% BSA and 0.05% Tween 20 in PBS at room temperature for 2 h. They were then probed at room temperature for 1 h with purified bovine antimelibiose antibodies (1 μg/ml) in PBS containing 1% BSA and 0.05% Tween 20. Melibiose was used as an inhibitor at a concentration of 10 mM. The remaining steps with affinity-purified, alkaline phosphatase-conjugated ant-bovine IgG whole molecule and BCIP-nitroblue tetrazolium substrate were the same as those described above.

RESULTS

The bovine anti-alpha galactosyl antibodies in serum were measured with an ELISA using melibiose (Galα1-6Glc)-BSA as an antigen (see Materials and Methods). The serum titration curves of seven serum samples, two pooled newborn, two pooled calf, two adult, and one pooled adult, are presented in Fig. 1. They were parallel to each other, suggesting that the measured antibodies reacted against the same epitope. Calf sample 2, a pooled serum sample, was used as the reference sample and included in all assays. The specificity of this assay was confirmed by inhibition assays with melibiose (see below).

Specificity of bovine anti-alpha galactosyl antibodies. Inhibition assays with various monosaccharides, disaccharides, and oligosaccharides were used to determine the specificity of the bovine anti-alpha galactosyl antibodies. The results are shown in Table 1 and Fig. 2. Based on the lowest concentrations showing a 50% reduction in OD, melibiose was the strongest inhibitor, followed equally by stachyose (Galα1-6Glcβ1-2Fru) and raffinose (Galα1-6Glcβ1-2Fru) and then almost equally by Galβ1-6Gal, Gal, and Galα1-2Gal (Table 1). Others, including Galα1-3Gal, Galα1-4Gal, and lactose (Galβ1-4Glc), exhibited only minor inhibition (Table 1). Since the Galα1-6Glc or Galα1-6Gal structure was present at the nonreducing end of stachyose or raffinose, these results suggest that these bovine anti-alpha galactosyl antibodies preferentially react with galactose in the α-1,6 linkage (Galα1-6Glc or Galα1-6Gal).

To compare the specificity of these bovine anti-alpha galactosyl antibodies with those of human antibodies, Galα1-3Gal–BSA was used as an antigen in a comparison with the melibiose (Galα1-6Glc)–BSA antigen. The assays with these two antigens were not standardized with respect to the exact amount of antigen used to coat each well but rather performed under the same conditions to provide a relative comparison. It was found that...
that the bovine serum reacted much more strongly to melibiose than to the Galα1-3Gal antigen, with an average OD (melibiose/Galα1-3Gal) ratio of 2.2 to 3.0 over a dilution range of 1:5 to 1:160 (Fig. 3A). This is consistent with the observation that Galα1-3Gal was a poor inhibitor of the bovine antibodies when reacting to melibiose-BSA (Table 1 and Fig. 2). On the other hand, human antibodies reacted with these two antigens to a similar degree, with an average OD (melibiose/Galα1-3Gal) ratio of 1.2 to 1.5 over the same dilution range (Fig. 3B).

Compared with bovine antibodies, the human antibodies exhibited a weaker reaction with the melibiose antigen (Fig. 3A and B) but a stronger reaction with Galα1-3Gal (Fig. 3A and B). These results further indicate that bovine anti-alpha galactosyl antibodies are distinct from those in humans with respect to antigen specificity. It seems that the specificity of bovine antibodies is more restricted to melibiose compared to that of human antibodies to Galα1-3Gal.

Presence of bovine anti-alpha galactosyl antibodies in different age groups. Twenty-seven bovine serum samples of three age groups were tested (Fig. 4). They included 3 pooled newborn (1 to 10 days old) samples, 5 pooled calf (<6 months old) samples, and 19 adult (>1 year old) samples. All of them, including those from the newborn, contained the antimelibiose antibodies. The antibody titers gradually increased from newborn to adult samples (Fig. 4), although the differences were not statistically significant. Titers in individual adult samples varied widely, with an average of 754 and a standard deviation of 546 (Fig. 4), although they exhibited a normal distribution (95% of the values were within the mean ± 2 standard deviations). Those at an age of 5 to 9 years (no. 12 to 16-1) exhibited the highest titers. It was noted that the titer of the freshly obtained sample (16-2) was fourfold lower than that of the one (16-1) obtained 4 years ago. By 1996, this cow was more than 10 years old.

Adult serum sample 2 had the lowest titer. In light of the lack of a known negative serum sample, a sugar inhibition assay was performed to ascertain that this sample was, indeed, positive. Melibiose at 3 mM produced a more than 50% inhibition of this sample, but lactose at 12 mM did not (data not shown). This confirms that this sample did contain the antimelibiose antibodies.

Only weak reactions were detected when the anti-bovine IgM (μ) chain conjugate was used; all sample ODs were less than twofold higher than the background. This suggests that the bovine antimelibiose antibodies are mainly of the IgG isotype.

Purification of bovine anti-alpha galactosyl antibodies. The anti-alpha galactosyl antibodies were purified by affinity chromatography using melibiose-agarose. Gel electrophoretic analysis of proteins eluted from the column with melibiose showed that the IgG heavy chain was the dominant band (Fig. 5, lane 1). The antibody heavy and light chains were identified by immunoblotting (Fig. 5, lanes 2 and 3). The IgM heavy chain was barely detectable, compared to the IgG heavy chain (Fig. 5). This observation is consistent with the results described above, suggesting that the bovine antimelibiose antibodies are mainly of the IgG isotype. The protein close to 200 kDa (Fig. 5, lanes 1 and 2) was most likely the undenatured IgG whole molecule, since it reacted with anti-IgG whole molecule antibody (Fig. 5, lane 2) but not with anti-IgM heavy (μ) chain antibody (Fig. 5, lane 3).

Out of 20 ml of the reference serum sample (calf sample 2), an average of 444 μg of antibodies was obtained in two separate experiments (444 and 475 μg), giving a concentration of 23 μg/ml in the serum. The titer (681) of calf sample 2 was below the average (713) of all samples, and so, most of the samples likely have a higher concentration of such antibodies.

Anti-alpha galactosyl IgA, IgG1, and IgG2. The anti-alpha galactosyl IgA, IgG1, and IgG2 antibodies were examined using 2 pooled newborn samples, 2 pooled calf samples, and 1 pooled adult sample along with 10 individual adult samples. The anti-alpha galactosyl antibody was primarily of the IgG1 isotype in all samples, except for two adult samples, 7 and 10, which had more IgG2 than IgG1 (Fig. 6A and B). However, it was noted that the IgG2 level was significantly increased in

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**TABLE 1. Inhibition of the reaction of bovine anti-alpha galactosyl antibodies to melibiose-BSA antigen by various sugars**

| Inhibitor | Concn (mM) at ≥50% inhibition | Expt 1a | Expt 2b |
|-----------|-------------------------------|---------|---------|
| Melibiose (Galα1-6Glc) | 0.023 | 0.047 |
| Stachyose (Galα1-6Glcα1-6Galβ1-2Fru) | 0.094 | 0.188 |
| Raffinose (Galα1-6Glcβ1-2Fru) | 0.188 | 0.094 |
| Galβ1-6Gal | 0.75 | 0.75 |
| Galactose | 0.75 | 0.75 |
| Galα1-2Gal | 0.75 | 1.5 |
| Galα1-3Gal | 3 | 3 |
| Galα1-4Gal | >6 | >6 |
| Lactose (Galβ1-4Glc) | >24 | >24 |
| Mannose | NTc | >24 |
| Glucose | >24 | >24 |
| Fucose | >24 | >24 |

a In experiment 1, calf serum sample 2 was used at a 1:80 dilution.

b In experiment 2, calf serum sample 2 was used at a 1:160 dilution.

NT, not tested.

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FIG. 2. Sugar inhibition assays. Calf serum sample 2 was used at a 1:80 dilution.
calves and adults compared to newborns ($P < 0.05$). In fact, the increase in IgG2 accounted for 81% of the total IgG increase in calves and adults. IgA was present in all of the samples, and its level was much higher in newborns than in calves or adults (Fig. 6A and B).

Reaction of purified bovine anti-alpha galactosyl antibodies with *S. agalactiae*. Many bacteria, including *Streptococcus* spp., are known to have immunoreactive terminal galactose residues in capsular polysaccharides (2, 20). *S. agalactiae* and group B streptococci are major causes of human neonatal infections (24) and also bovine mastitis (16). To determine if the bovine anti-alpha galactosyl antibodies react with these bacteria and others, three American Type Culture Collection *S. agalactiae* strains (serotypes II, IV, and V) and a bovine *S. agalactiae* clinical isolate (5247) were tested in a dot blot test along with strains of *E. coli*, *S. aureus*, and *P. aeruginosa*. The type II *S. agalactiae* strain is an isolate from a bovine with mastitis, whereas the type IV and V strains are from humans. The results of the dot blot tests showed that these bovine antibodies reacted with the type II bovine strain and the clinical bovine isolate (5247) (Fig. 7A). No reaction was detected with the type IV or V human strain (Fig. 7A) or with the *E. coli*, *S. aureus*, and *P. aeruginosa* strains tested (data not shown). The reaction with bovine strains was inhibited by melibiose (Fig. 7B).

**DISCUSSION**

The present experiments demonstrated that cattle possess an anti-alpha galactosyl antibody with a serum concentration.
close to that of human anti-Galα1-3Gal antibodies (20 to 100 μg/ml). However, the specificity of these bovine antibodies is distinctly different from that of human antibodies. The bovine anti-alpha galactosyl antibodies preferentially reacted with the galactose in α1-6 linkage, i.e., Galα1-6Glc or Galα1-6Gal.

Stachyose and raffinose are the second strongest inhibitors (next to melibiose) (Table 1). It is not known why stachyose (Galα1-6Galα1-6Glcβ1-2Fru) and raffinose (Galα1-6Glcβ1-2Fru) are less efficient inhibitors than melibiose, especially the latter, which has a structure identical to that of melibiose (Galα1-6Glc) at the nonreducing end. It may be that the third sugar affects the binding; i.e., the third sugar found in stachyose and raffinose may not be the right one for the optimal binding of these antibodies. Alternatively, it could be due to the fact that larger molecules are simply less efficient competitors than the smaller ones bearing the same functional domain. Similar observations have been made with human anti-alpha galactosyl antibodies (35).

Galβ1-6Gal and Galα1-2Gal were moderate inhibitors (Table 1), suggesting that a significant reaction may also be found with these two structures. On the other hand, Galα1-3Gal, Galα1-4Gal, and lactose (Galβ1-4Glc) were weak inhibitors. Together, it seems that these bovine antibodies favor the linkage positions more than the anomer configuration of the linkage since Galβ1-6Gal is a more efficient inhibitor than Galα1-3Gal and Galα1-4Gal.

The lack of a strong reaction of bovine anti-alpha galactosyl antibodies to the Galα1-3Gal structure is consistent with the fact that the Galα1-3Gal epitope is a self antigen in animals, including cattle, due to the presence of alpha (1-3) galactosyltransferase (13). By the same token, cattle might lack the Galα1-6Glc or Galα1-6Gal structure in their tissues; i.e., they might lack an alpha (1-6) galactosyltransferase. Whether humans have such an enzyme also remains to be determined, although the Galα1-6Gal structure has been reported in human tumor cells (10, 15).

The origin of these bovine antibodies is not known. They could develop in response to any environmental agents bearing this epitope. The positive reaction of these antibodies with bovine S. agalactiae suggests that responses to bacteria, either pathogenic or nonpathogenic, can be one of the sources, although a positive reaction itself does not necessarily prove the possession of an identical epitope (Galα1-6Glc or Galα1-6Gal) by the bacteria. S. agalactiae is a primary pathogen of bovine mastitis and is of major economic concern in the dairy industry (16). It is also the leading cause of human neonatal mortality and morbidity (24). Although all serotypes can be found in both human and animals, serotype II is one of the most frequently isolated serotypes (II and III) in bovine mastitis cases (12, 31).

Structures of capsular polysaccharides of bovine S. agalactiae have not been elucidated. Studies on the human strains of all six serotypes showed that there is no terminal α(1-6)-linked galactose residue present in the capsular polysaccharides (11, 33). However, terminal galactose residues in β1-6 linkage are present in the type II strain (11). Galβ1-6Gal was found to be a moderate inhibitor of these bovine anti-alpha galactosyl an-
tibodies. Thus, it seems consistent with the finding that these bovine antibodies reacted negatively with the human type IV and V S. agalactiae strains but positively with the bovine type II strain. Studies with S. cricetus and S. sobrinus have also shown that only certain serotypes possess the terminal galactose residues in a particular linkage (2, 20).

Bovine antimelibiose antibodies are clearly present in newborns and are most likely from the mother’s colostrum. In cattle, there is no evidence of antimelibiose antibodies in sera from patients with germ cell tumors. Cancer Res. 47:2288–2294.

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REFERENCES

1. Avila, J. L., and A. Bretana. 1993. Alpha-galactosyl epitope on Trypanosoma cruzi, Leishmania mexicana and L. braziliensis. Ultrastructural localization and possible role of antibodies against this epitope. Acta Microsc. 2:43–54.

2. Brown, T. A., and A. S. Bleilevish. 1979. Chemical, immunochemical, and structural studies of the cross-reactive antigens of Streptococcus mutans AHT and B13. Infect. Immun. 24:326–336.

3. Butler, J. E. 1986. Biochemistry and biology of ruminant immunoglobulins. Prog. Vet. Microbiol. Immun. 2:1–153.

4. Estes, D. M., W. C. Brown, and A. Hirano. 1998. CD40 ligand-dependent signaling of bovine B lymphocyte development and differentiation. Vet. Immunol. Immunopathol. 63:15–20.

5. Galili, U., E. A. Rachmilewitz, A. Peleg, and I. Flechner. 1984. A unique natural human IgG antibody with anti-alpha-galactosyl specificity. J. Exp. Med. 160:1519–1531.

6. Galili, U., B. A. Macher, J. Buehler, and S. B. Shohet. 1985. Human natural anti-alpha-galactosyl IgG. II. The specific recognition of alpha (1-3)-linked galactose residues. J. Exp. Med. 162:573–582.

7. Galili, U., M. R. Clark, S. B. Shohet, J. Buehler, and B. A. Macher. 1987. Evolutionary relationship between the natural anti-Gal antibody and the Gal alpha 1-3 Gal epitope in primates. Proc. Natl. Acad. Sci. USA 84:1369–1373.

8. Galili, U., R. E. Mandrell, R. M. Hamadeh, S. B. Shohet, and J. M. Griffliss. 1988. Interaction between human natural anti-alpha-galactosyl immunoglobulin G and bacteria of the human flora. Infect. Immun. 56:1730–1737.

9. Galili, U., S. B. Shohet, E. Kobrin, C. L. Stults, and B. A. Macher. 1988. Man, apes, and Old World monkeys differ from other mammals in the expression of alpha-galactosyl epitopes on nucleated cells. J. Biol. Chem. 263:17755–17762.

10. Gollogly, L., and V. Castronovo. 1996. A possible role for the alpha-1-3 galactosyl epitope and the natural anti-gal antibody in oncogenesis. Neoplasia 42:265–268.

11. Jennings, H. J., and R. A. Pon. 1996. Polysaccharides and glycoconjugates as human vaccines, p. 443–479. In S. Dumitru (ed.), Polysaccharides in medical applications. Marcel Dekker, Inc., New York, N.Y.

12. Jensen, N. E. 1985. Epidemiological aspects of human/animal interrelationships in GBS. J. Infect. Chemother. 35:40–48.

13. Joziassie, D. H., J. H. Shaper, D. H. Van Den Eijnden, A. H. Van Tunen, and N. L. Shaper. 1989. Bovine alpha 1-3-galactosyltransferase: isolation and possible role of antibodies against this epitope. Acta Microsc. 35:49–58.

14. Joziassie, D. H., J. H. Shaper, E. W. Jabs, A. H. Van Tunen, and N. L. Shaper. 1991. Characterization of alpha-1-3-galactosyltransferase homologue on human chromosome 12 that is organized as a processed pseudogene. J. Biol. Chem. 266:6991–6998.

15. Kawata, M., S. Sekiya, H. Takamizawa, T. Muramatsu, and K. Okumura. 1987. Molecular properties of F9 embryoglycan recognized by a unique antibody in sera from patients with germ cell tumors. Cancer Res. 47:2288–2294.

16. Keefe, G. P. 1997. Streptococcus agalactiae mastitis: a review. Can. Vet. J. 38:429–437.
17. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685.

18. Larsen, R. D., V. P. Rajan, M. Ruff, J. Kukowska-Latallo, R. D. Cummings, and J. B. Lowe. 1989. Isolation of a cDNA encoding a murine UDPgalactose: beta-D-galactosyl, 4-N-acetyl-D-glucosaminide alpha 1,3-galactosyltransferase: expression cloning by gene transfer. Proc. Natl. Acad. Sci. USA 86:8227–8231.

19. Larsen, R. D., C. A. Rivera-Marrero, L. K. Ernst, R. D. Cummings, and J. B. Lowe. 1990. Frameshift and nonsense mutations in a human genomic sequence homologous to a murine UDPGA1: beta-D-Gal(1, 4)-GlcNAc-alpha 1,3-galactosyltransferase cDNA. J. Biol. Chem. 265:7055–7061.

20. Ota, F., H. Kato, and K. Fukui. 1987. Immunological study of cross-reactive polysaccharide antigens (types a, d, and h) of oral Streptococcus spp. with monoclonal antibodies. Infect. Immun. 55:266–268.

21. Rother, R. P., W. L. Fodor, J. P. Springhorn, C. W. Birks, E. Setter, M. S. Sandrin, S. P. Squinto, and S. A. Rollins. 1995. A novel mechanism of retrovirus inactivation in human serum mediated by anti-alpha-galactosyl natural antibody. J. Exp. Med. 182:1345–1355.

22. Rother, R. P., and S. P. Squinto. 1996. The alpha galactosyl epitope: a sugar coating that makes viruses and cells unpalatable. Cell 86:185–188.

23. Sandrin, M. S., and I. F. McKenzie. 1994. Gal alpha (1,3)Gal, the major xenoantigen(s) recognised in pigs by human natural antibodies. Immunol. Rev. 141:169–190.

24. Schuchat, A. 1993. Epidemiology of group B streptococcal disease in the United States: shifting paradigms. Clin. Microbiol. Rev. 11:497–513.

25. Srivivasan, A., Y. Ni, and I. Tizard. 1999. Specificity and prevalence of natural bovine antimannan antibodies. Clin. Diagn. Lab. Immunol. 4946–952.

26. Sugii, S., and Y. Hirota. 1990. Isolation and hemagglutinating activities of bovine immunoglobulins reactive with melibiose. Jpn. J. Vet. Sci. 52:939–945.

27. Sugii, S., and Y. Hirota. 1993. Identification and characterization of the major carbohydrate-binding proteins in chicken serum as immunoglobulins. J. Vet. Med. Sci. 55:125–128.

28. Takeuchi, Y., C. D. Porter, K. M. Strahan, A. F. Preece, K. Gustafsson, F. L. Cosset, R. A. Weiss, and M. K. Collins. 1996. Sensitization of cells and retroviruses to human serum by (alpha 1-3) galactosyltransferase. Nature 379:85–88.

29. Tao, W., M. J. Corbett, and W. Pickett. 1995. Monomeric bovine IgG2 is a potent stimulus for bovine neutrophils. J. Leukoc. Biol. 58:203–208.

30. Travassos, L. R., and I. C. Almeida. 1993. Carbohydrate immunity in American trypanosomiasis. Springer Semin. Immunopathol. 15:183–243.

31. Van Den Heever, L. W., and M. Erasmus. 1980. Group B streptococcus—comparison of Streptococcus agalactiae isolated from humans and cows in the Republic of South Africa. J. South Afr. Vet. Assoc. 51:93–100.

32. Welsh, R. M., C. L. Odonnell, D. J. Reed, and R. P. Rother. 1998. Evaluation of the Galα1-3Gal epitope as a host modification factor eliciting natural humoral immunity to enveloped viruses. J. Virol. 72:4650–4656.

33. Wessels, M. R., J. L. DiFabio, V. J. Benedi, D. L. Kasper, F. Michon, J. R. Brisson, J. Jelinkova, and H. J. Jennings. 1991. Structural determination and immunochemical characterization of the type V group B streptococcus capsular polysaccharide. J. Biol. Chem. 266:6714–6719.

34. Wiener, A. S. 1951. Origin of naturally occurring hemagglutinins and hemolysins. J. Immunol. 66:287–295.