ORIGINAL ARTICLE

Early-Childhood Membranous Nephropathy Due to Cationic Bovine Serum Albumin

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ABSTRACT

BACKGROUND

The M-type phospholipase A_2 receptor (PLA_2R) was recently identified as a candidate antigen in 70% of cases of idiopathic membranous nephropathy, a common form of the nephrotic syndrome. The nature of antigens involved in other idiopathic and secondary membranous nephropathies remains unclear.

METHODS

We searched for antibodies against bovine serum albumin and circulating bovine serum albumin by means of enzyme-linked immunosorbent assay and Western blotting in serum specimens obtained from 50 patients with membranous nephropathy and 172 controls. The properties of immunopurified circulating bovine serum albumin obtained from serum specimens were analyzed with the use of two-dimensional sodium dodecyl sulfate–polyacrylamide-gel electrophoresis. We detected bovine serum albumin in glomerular deposits and analyzed the reactivity of eluted IgG.

RESULTS

Eleven patients, including four children, had high levels of circulating anti–bovine serum albumin antibodies, of both the IgG1 and IgG4 subclasses. These patients also had elevated levels of circulating bovine serum albumin, without an increase in circulating immune complex levels. Bovine serum albumin immunopurified from the serum specimens of these four children migrated in the basic range of pH, whereas the bovine serum albumin from adult patients migrated in neutral regions as native bovine serum albumin. Bovine serum albumin was detected in subepithelial immune deposits only in the children with both high levels of cationic circulating bovine serum albumin and bovine serum albumin–specific antibodies, and it colocalized with IgG in the absence of PLA_2R. IgG eluted from such deposits was specific for bovine serum albumin.

CONCLUSIONS

Some patients with childhood membranous nephropathy have both circulating cationic bovine serum albumin and anti–bovine serum albumin antibodies. Bovine serum albumin is present in immune deposits, suggesting that cationic bovine serum albumin is pathogenic through binding to the anionic glomerular capillary wall and in situ formation of immune complexes, as shown in experimental models.

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Membranous nephropathy is the most common cause of the nephrotic syndrome in adults but is rare in children.[1,2] The central pathogenesis involves the formation of subepithelial immune deposits that are responsible for functional impairment of the glomerular capillary wall.[1,3] Two major antigens have been identified. The first is neutral endopeptidase, the alloantigen involved in neonatal cases of membranous nephropathy,[4] and the second is the M-type phospholipase A_2 receptor (PLA_2R), which has been identified in idiopathic membranous nephropathy.[5] Idiopathic membranous nephropathy is considered an autoimmune disease, whereas secondary forms involve exogenous antigens such as viral, bacterial, and tumoral antigens.[6] It is likely that a growing number of cases of "idiopathic" membranous nephropathy will be reclassified as secondary once nonglomerular antigens are identified.

Epidemiologic surveys have identified nutritional elements as risk factors for the development of autoimmunity in genetically susceptible persons.[7-9] Bovine serum albumin is one of the cow's milk and beef proteins that can escape from the intestinal barrier and thus induce formation of anti–bovine serum albumin antibodies. Today, food ingredients are subjected to a variety of processing conditions that may induce modification of their proteins, which could affect the digestion of these proteins and allow their passage into the bloodstream.[10-12]

We report on a mechanism for childhood membranous nephropathy involving anti–bovine serum albumin antibodies and a modified food-derived antigen, cationic bovine serum albumin, which appears to become planted in the anionic glomerular capillary wall, thus inducing in situ formation of immune complexes.

Methods

Patients

We assessed a consecutive cohort of 9 children and 41 adults with idiopathic membranous nephropathy; all of these patients underwent biopsy between 2004 and 2009. These patients lacked features of secondary membranous nephropathy. Their clinical characteristics are listed in Table 1 in the Supplementary Appendix (available with the full text of this article at NEJM.org). No manifestation of a cow's milk allergy was observed in any of the patients. Serum specimens were also obtained from 63 age-matched patients with other glomerular diseases and 109 controls without proteinuria (Table 2 in the Supplementary Appendix). A human subjects committee approved the study, and written informed consent was obtained from all adults and from the parents of all the children; in addition, assent was obtained from children older than 10 years of age.

IgG Antibodies to Bovine Serum Albumin and Circulating Immune Complexes

Circulating antibodies were detected on enzyme-linked immunosorbent assay (ELISA) plates coated with bovine serum albumin (Sigma). Circulating immune complexes containing C1q or C3d were detected with the use of ELISA kits (Quidel).

Peptide-Based ELISA

A panel of selected peptides (Mimotopes) was purchased. The peptide solutions were covalently immobilized in the wells, and diluted (1:100) serum specimens were used for assays. IgG antibodies to peptides were detected with the use of alkaline phosphatase–conjugated antihuman IgG (Sigma).

Circulating Bovine Serum Albumin

Bovine serum albumin was detected in patients' serum specimens with the use of a bovine albumin ELISA kit (Alpha Diagnostic International). Bovine serum albumin was immunopurified from the serum specimens obtained from patients and controls or from bovine serum by means of affinity chromatography with the use of anti–bovine albumin agarose (Sigma). Immunopurified bovine serum albumin was also analyzed by means of two-dimensional electrophoresis. The first dimension was run on immobilized pH gradient ready strips at a pH of 3 to 10 (BioRad), and the second was run on 8% sodium dodecyl sulfate–polyacrylamide-gel electrophoresis (SDS-PAGE). The separated proteins were blotted on polyvinylidene fluoride (PVDF) membrane, and the position of bovine serum albumin was determined with chicken horseradish peroxidase–conjugated anti–bovine serum albumin antibodies (GeneTex).

Western Blotting and Elution of IgG

Bovine serum albumin and human serum albumin were electrophoresed and transferred to PVDF membranes, according to standard protocols. Detection antibodies were peroxidase-conjugated goat antihuman antibodies (Chemicon). IgG sub-

classes were determined with the use of mouse monoclonal antihuman IgG1, IgG2, IgG3, and IgG4 antibodies (developed at and sold by Birmingham University, United Kingdom), followed by peroxidase-conjugated sheep antimouse IgG antibodies (GE Healthcare). Immunoglobulins were acid-eluted from the cores of kidney-biopsy specimens obtained from patients with membranous nephropathy. The eluted IgG was used to immunoblot the bovine serum albumin and human serum albumin directly.

IMMUNOHISTOLOGIC ANALYSIS

We analyzed cryosections or paraffin-embedded sections of normal human kidney and biopsy specimens obtained from the patients with membranous nephropathy and from patients with other glomerular diseases. We used rabbit polyclonal anti–bovine serum albumin antibodies (Invitrogen) to detect bovine serum albumin in cryosections, and we used rabbit polyclonal anti-PLA_2R antibodies (Atlas Antibodies) followed by goat Alexa 488–conjugated anti–rabbit Fab IgG antibodies (Molecular Probes) to detect PLA_2R in paraffin-embedded sections. Colocalization of bovine serum albumin and IgG was analyzed by means of confocal microscopy. Cryosections of the biopsy specimens were first incubated with rabbit polyclonal anti–bovine serum albumin antibodies (Invitrogen), then with goat Alexa 488–conjugated anti–rabbit Fab IgG antibodies and goat Alexa 568–conjugated anti–human IgG antibodies (Molecular Probes). The cryosections were also stained with mouse monoclonal anti–human IgG1, IgG2, IgG3, and IgG4 antibodies followed by rabbit Alexa 488–conjugated antimouse antibodies. Sections were examined under a confocal microscope (Leica TCS SP2) and analyzed with the use of Leica Confocal Software, version 2.61 (Cellular Imaging Platform, Institut Fédératif de Recherche 65).

STATISTICAL ANALYSIS

The nonparametric Mann–Whitney U test with Bonferroni's correction was used for comparison of levels of anti–bovine serum albumin antibodies or circulating bovine serum albumin in patients with membranous nephropathy as compared with control subjects in each age group. P values of less than 0.008 were considered to indicate statistical significance; this value was chosen because six comparisons were made, and the alpha value of each comparison was 0.05 divided by the number of comparisons.

RESULTS

ANTI–BOVINE SERUM ALBUMIN ANTIBODIES AND EPITOPE MAPPING

Because anti–bovine serum albumin antibodies are common in the general population,[13] we investigated whether they could be related to the pathogenesis of membranous nephropathy and recognize specific epitopes, as shown in rheumatoid arthritis[14] and multiple sclerosis.[15] High levels of anti–bovine serum albumin antibodies were detected in 4 of 5 consecutive children in the first age group (<5 years) and in 7 of 41 consecutive adults with membranous nephropathy (Fig. 1A). In the first two age groups (<5 years and 5 to 16 years), control subjects with other diseases had lower levels of anti–bovine serum albumin antibodies than patients with membranous nephropathy. In the first age group (<5 years), the controls without proteinuria had lower levels of antibodies than patients with membranous nephropathy. Two controls in the older age groups (5 to 16 years and >16 years) had high levels of anti–bovine serum albumin antibodies. In serum samples obtained from the patients and controls with the highest levels of anti–bovine serum albumin antibodies detected by means of ELISA, IgG showed reactivity with bovine serum albumin, but not with human serum albumin, as detected by means of Western blotting (Fig. 1B). Anti–bovine serum albumin antibodies were mainly of the IgG1 and IgG4 subclasses, with a predominance of either IgG1 or IgG4 (Fig. 1C). In contrast to the patients with IgE-mediated bovine serum albumin allergy, those with membranous nephropathy did not have detectable increases in the level of anti–bovine serum albumin IgE (data not shown).

We hypothesized that bovine serum albumin–specific antibodies reacted primarily with sequential epitopes in which the amino acid sequences differ greatly between bovine serum albumin and human serum albumin. Fourteen peptide candidate epitopes corresponding to dissimilarity regions were synthesized (Table 3 in the Supplementary Appendix). All responses to bovine serum albumin in the patients with membranous nephropathy predominantly targeted the bovine serum albumin peptide 147-161, whereas controls with the higher level of anti–bovine serum albumin antibodies had a broader spectrum of reactivity toward the synthesized peptides. Peptide 147-161 contains two linear epitopes that are not

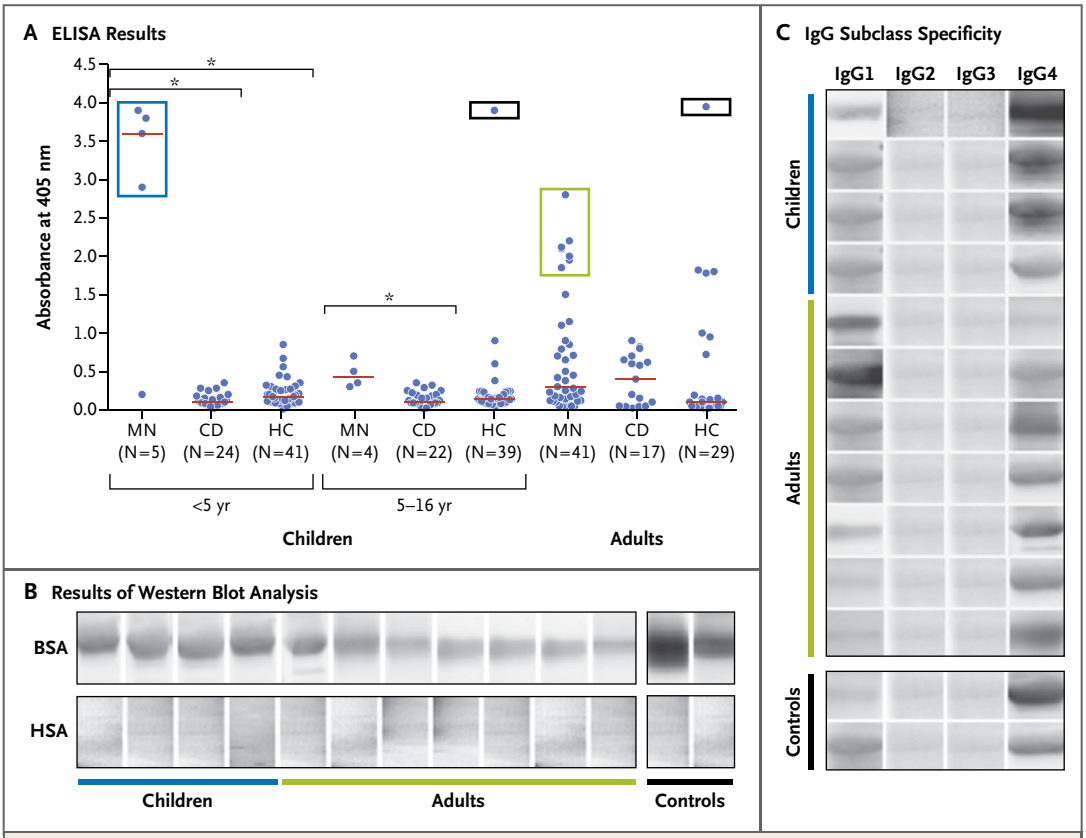


Figure 1. Characterization of Circulating Anti–Bovine Serum Albumin Antibodies.

Panel A shows the absorbance values of a specific enzyme-linked immunosorbent assay (ELISA), with higher absorbance indicating higher antibody levels, to assess antibodies to bovine serum albumin (BSA) in serum specimens from patients with membranous nephropathy (MN), patients with other types of glomerular diseases (controls with disease [CD]), and healthy controls (HC). In children younger than 5 years, the nonparametric Mann–Whitney U test with Bonferroni's correction showed a significant difference between the absorbance values in the serum specimens from patients with MN and the values in specimens from both controls with disease and healthy controls. In the children between 5 and 16 years of age, a significant difference was observed only between patients with MN and controls with disease. Blue circles represent individual patients, and horizontal lines medians for each group. The asterisk indicates a P value of less than 0.008, which was considered to be statistically significant. Panel B shows the results of Western blot analysis of IgG reactivity with BSA and human serum albumin (HSA) in serum specimens from patients with MN and from controls. Reactivity of serum was detected with the use of anti–human IgG antibody. Panel C shows the IgG subclass specificity to BSA. BSA was blotted with serum samples obtained from patients indicated by the boxes in Panel A, followed by mouse antibodies specific for each human IgG subclass. The colors of the bars on the x axis in Panel B and the y axis in Panel C correspond to the colors of the boxes in Panel A.

present in human serum albumin (Fig. 1A, 1B, and 1C in the Supplementary Appendix).

IDENTIFICATION AND CHARACTERIZATION OF CIRCULATING BOVINE SERUM ALBUMIN

Because trypsin specifically cleaves proteins on the carboxyl side of Arg and Lys residues, the bovine serum albumin peptide 147-161 should be broken down in the gut. Therefore, we speculated that in pathologic conditions, a substantial amount of the bovine serum albumin protein was not digested or was only partially digested. The four children with membranous nephropathy and high levels of anti–bovine serum albumin antibodies also had high levels of circulating bovine serum albumin (Fig. 2A). Among the seven adults with membranous nephropathy and high levels of anti–bovine serum albumin antibodies, four also had elevated levels of circulating bovine serum albumin, albeit in a lower range than the levels in the four children (Fig. 2A). The two controls with high levels of anti–

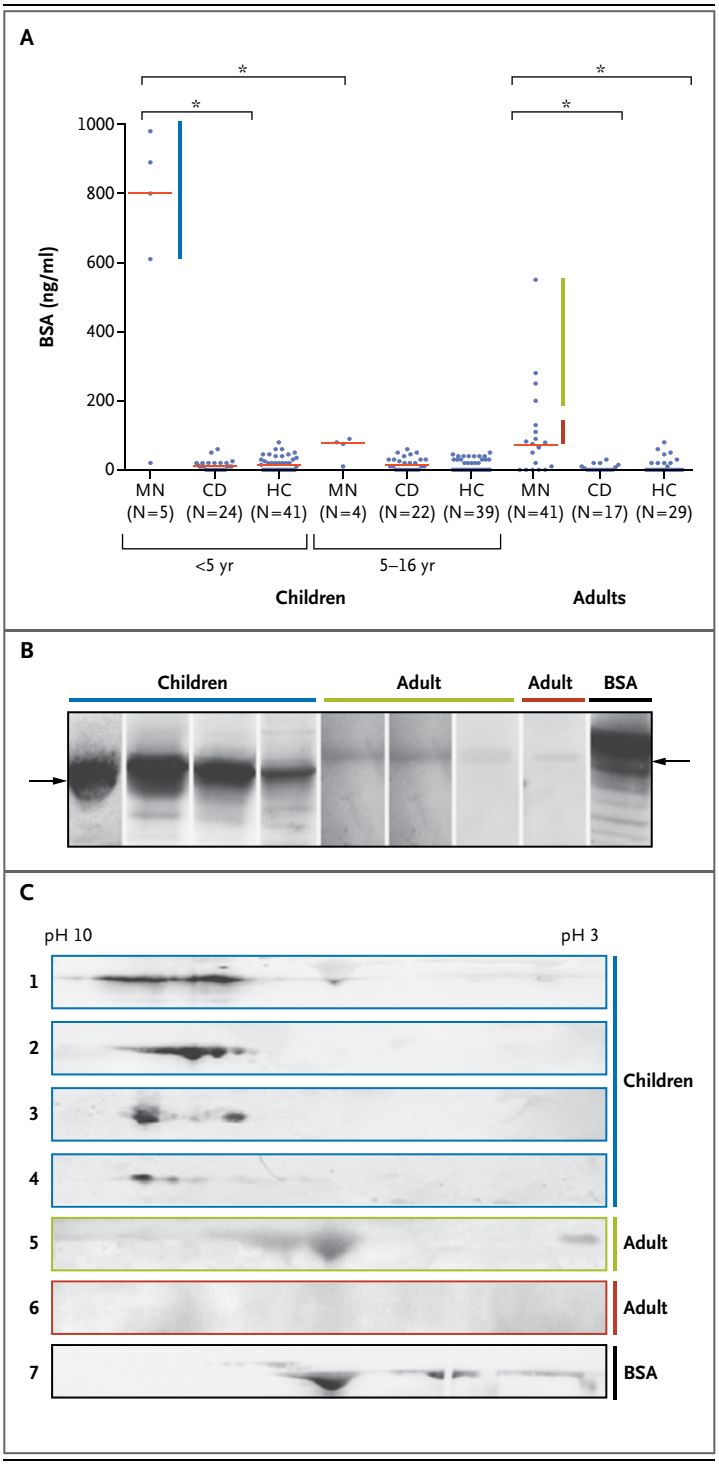


Figure 2. Detection and Characterization of Circulating Bovine Serum Albumin (BSA).

Panel A shows the amount of circulating BSA assessed by means of enzyme-linked immunosorbent assay in serum specimens from patients with membranous nephropathy (MN), patients with other types of glomerular diseases (controls with disease [CD]), and healthy controls (HC). All patients and controls were divided into three groups according to age: younger than 5 years, 5 to 16 years, and adult. The nonparametric Mann–Whitney U test with Bonferroni's correction showed a significant difference in the levels of BSA between patients with MN and both controls with disease and healthy controls in the youngest and oldest age groups. The four children with MN and high levels of anti–bovine serum albumin antibodies also had high levels of circulating BSA (blue bar). Blue circles represent individual patients, and horizontal lines medians for each group. The asterisk indicates a P value of less than 0.008, which was considered to be statistically significant. Panel B shows the results of immunoblot analysis of BSA immunopurified from specimens obtained from patients (lanes 1 through 8) and of albumin immunopurified from bovine serum (lane 9). The arrows indicate the migration of the native BSA (right) and the circulating BSA in children with MN (left). Panel C shows the results of two-dimensional electrophoresis and immunoblot of affinity-purified BSA from serum specimens obtained from children (lanes 1 through 4) and adult patients (lanes 5 and 6) and of albumin immunopurified from bovine serum (lane 7). The colors of the horizontal bars in Panel B and the boxes in Panel C correspond to the vertical bars of the same colors in Panel A. Only representative samples for two individual adult patients are shown. The position of BSA was determined with horseradish peroxidase–conjugated anti-BSA antibodies.

bovine serum albumin antibodies had very low levels of circulating bovine serum albumin.

The apparent molecular weight of the bovine serum albumin reactive antigen was assessed by means of SDS-PAGE after immunopurification from serum specimens obtained from the patients with membranous nephropathy. Bovine serum albumin antigen migrated slightly faster than the native bovine serum albumin, which was immunopurified under the same conditions (Fig. 2B). Positively charged, cationic proteins can attach to the glomerular basement membrane and serve as a target for in situ immune-complex formation.[16-18] The cationic form of bovine serum albumin, but not the native form, which is slightly anionic, induced membranous nephropathy in various animal models.[16-18] Therefore, we used two-dimensional SDS-PAGE to analyze the bovine serum albumin immunopurified from patients' serum specimens obtained from the patients with membranous nephropathy. Bovine serum albumin circulating in the children with membranous nephropathy migrated in the basic range of pH (lanes 1 through 4 in Fig. 2C), whereas native bovine serum albu-

min migrated in neutral or slightly acidic regions (lane 7 in Fig. 2C). In contrast, in the adults with membranous nephropathy, immunopurified bovine serum albumin migrated as native bovine serum albumin (lane 5 in Fig. 2C) or was below the limit of detection (lane 6 in Fig. 2C).

Despite the presence of high levels of circulating bovine serum albumin and anti–bovine serum albumin antibodies, we did not detect significant amounts of complement-binding circulating immune complexes with the use of two different assays (CIC-C1q enzyme immunoassay, <4 μg per milliliter; CIC-Raji enzyme immunoassay, <10 μg per milliliter).

COLOCALIZATION OF BOVINE SERUM ALBUMIN AND IgG IN IMMUNE DEPOSITS AND ANTI–BOVINE SERUM ALBUMIN ACTIVITY OF ELUTED IgG

We searched for bovine serum albumin deposits in glomeruli in the 7 patients with high levels of anti–bovine serum albumin antibodies and circulating bovine serum albumin and in 15 additional patients with idiopathic membranous nephropathy who did not have circulating bovine serum albumin, irrespective of the presence or absence of anti–bovine serum albumin antibodies, for whom kidney-biopsy specimens were available (Table 4 in the Supplementary Appendix). Subepithelial granular deposits of bovine serum albumin were detected only in children who had both high levels of circulating cationic bovine serum albumin and bovine serum albumin–specific antibodies (Fig. 3A, and Table 4 in the Supplementary Appendix). No staining was seen in biopsy specimens obtained from patients with idiopathic membranous nephropathy who did not have circulating cationic bovine serum albumin, irrespective of the presence or absence of anti–bovine serum albumin antibodies (Fig. 3B and 3C). In patients with positive staining for bovine serum albumin, there was no M-type PLA_2R in immune deposits (Fig. 3D, and Table 4 in the Supplementary Appendix). However, PLA_2R was detected in 14 of the 20 biopsy specimens from patients without bovine serum albumin deposits (Fig. 3E, and Table 4 in the Supplementary Appendix), whereas the 6 remaining specimens that were negative for bovine serum albumin (Fig. 3C) were also negative for PLA_2R (Fig. 3F). Normal kidney tissue and biopsy specimens from patients with other nephropathies were negative for bovine serum albumin (Fig. 3G, 3H, and 3I, and Fig. 2A through 2D in the Supplementary Appendix). These overall results strongly suggest that four of the children had membranous nephropathy caused by circulating cationic bovine serum albumin. Detailed characteristics of these four children are provided in Tables 1, 5, 6, and 7 in the Supplementary Appendix.

Bovine serum albumin and IgG were colocalized in a fine granular pattern in many areas of the outer aspect of the capillary wall (Fig. 4A, 4B, and 4C). Quantitative analysis of the fluorescence showed a complete superimposition of the two signals (Fig. 3A and 3B in the Supplementary Appendix). The specificity of staining for bovine serum albumin was assessed in double-labeling confocal studies by preincubating anti–bovine serum albumin antibodies with pure bovine serum albumin (Fig. 4D) and by using anti–human serum albumin antibodies instead of anti–bovine serum albumin antibodies (Fig. 4F). A biopsy specimen also showed abundant subepithelial deposits of the membrane-attack complex C5b–C9 (Fig. 4G).

We eluted IgG from the biopsy specimens of one patient with membranous nephropathy who had bovine serum albumin glomerular deposits and four patients with membranous nephropathy who did not have bovine serum albumin deposits. Reactivity of IgG was analyzed by means of Western blotting with bovine serum albumin or human serum albumin. Figure 4H shows the presence of anti–bovine serum albumin IgG4 and IgG1 only with bovine serum albumin deposits; no reactivity was found with human serum albumin. These results are in keeping with the predominance of IgG1 and IgG4 subclasses in subepithelial deposits (Fig. 4I), a characteristic feature of membranous nephropathy,[19] and with the IgG subclass reactivity profile observed in serum (Fig. 1C).

ASSOCIATION WITH DISEASE ACTIVITY

All four children with circulating bovine serum albumin–related membranous nephropathy underwent a complete or partial remission (Table 7 in the Supplementary Appendix). We analyzed serum specimens collected serially from one child. High levels of anti–bovine serum albumin IgG4 and IgG1 antibodies (Fig. 5A) and circulating bovine serum albumin (Fig. 5B) were observed when there was clinically significant disease activity, as assessed on the basis of urinary protein levels. During remission, the levels of circulating bovine serum albumin and anti–bovine serum albumin antibodies were substantially decreased. The findings in two other children are shown in Fig. 4 and 5 in the Supplementary Appendix.

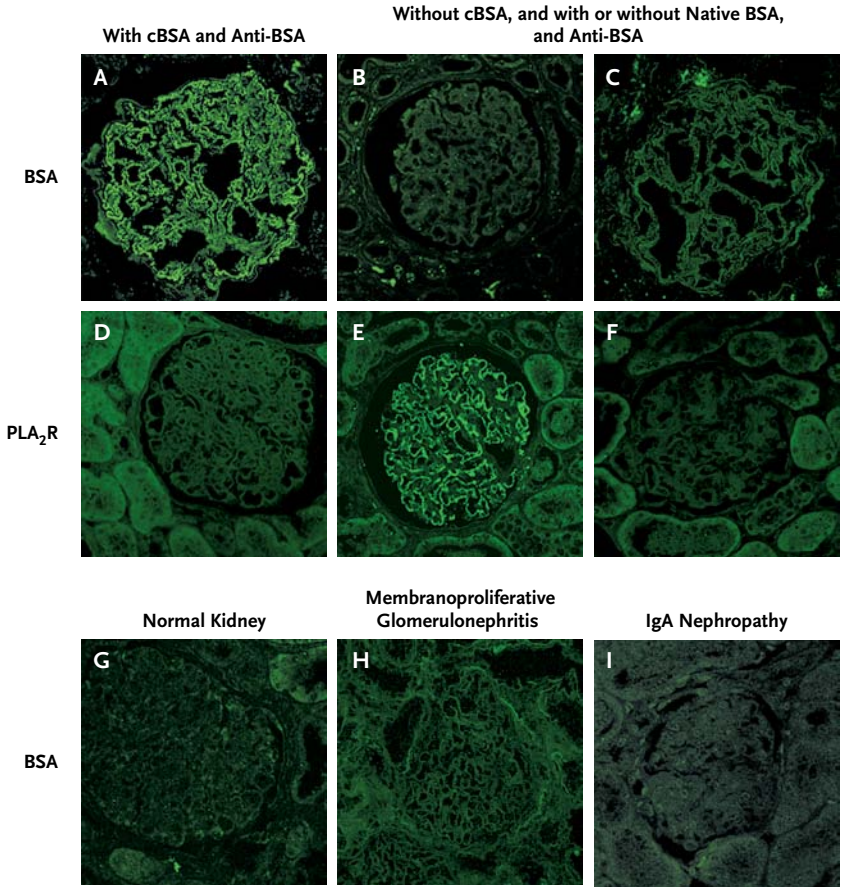


Figure 3. Expression of Bovine Serum Albumin (BSA) and M-Type Phospholipase A2 Receptor (PLA2R) in Biopsy Specimens.

Panel A shows representative staining of a biopsy specimen from a patient with circulating cationic bovine serum albumin (cBSA) and anti–bovine serum albumin antibodies (anti-BSA). BSA was detected in immune deposits. Panels B and C show representative immunostaining of biopsy specimens from patients without cBSA and with or without native BSA and anti-BSA. In the 20 tested biopsy specimens from these patients, BSA was not detected. PLA_2R was absent in glomerular immune deposits in a biopsy specimen from a patient with cBSA and anti-BSA (Panel D). The deposits were positive for PLA_2R in 14 BSA-negative samples (Panel E), and the 6 remaining biopsy specimens were also negative for PLA_2R (Panel F). The specimens shown in Panels A, B, and C are cryostat sections of kidney. The specimens shown in Panels D, E, and F are paraffin sections from the same patients. Staining for BSA is also shown in specimens from a patient with a normal kidney (Panel G), a patient with membranoproliferative glomerulonephritis (Panel H), and a patient with IgA nephropathy (Panel I).

DISCUSSION

We found a distinct form of membranous nephropathy in children 5 months to 2.3 years of age whose presentations were otherwise typical of idiopathic membranous nephropathy. These patients had both high levels of anti–bovine serum albumin antibodies of IgG1 and IgG4 subclasses and circulating cationic bovine serum albumin. Bovine serum albumin was colocalized with IgG in subepithelial immune deposits. IgG1 and IgG4 eluted from kidney-biopsy specimens had reactivity to bovine serum albumin. These data strongly suggest that in these patients, cationic bovine serum albumin became implanted in the anionic glomerular capillary wall, which led to the deposition of anti–bovine serum albumin IgG. These cases appear to be a human counterpart of experimental models in which the administration of cationic bovine serum albumin resulted in an-

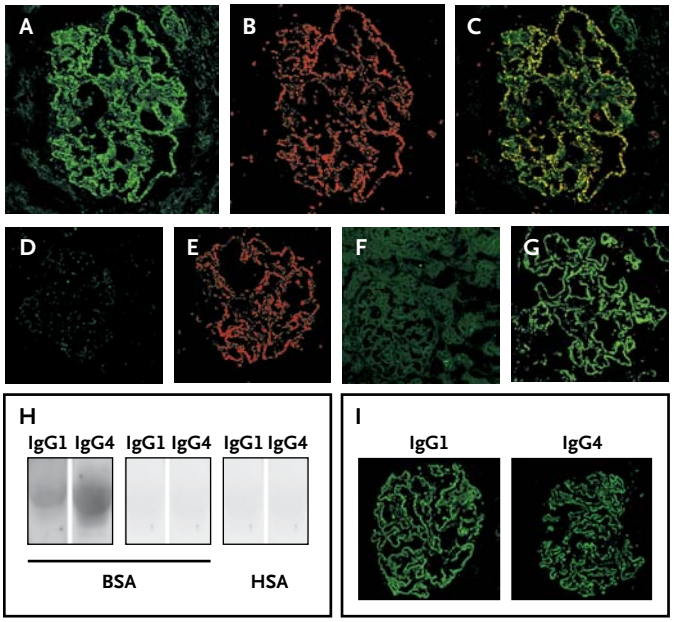


Figure 4. Colocalization of Bovine Serum Albumin (BSA) and IgG in Immune Deposits and Reactivity of Eluted IgG.

Panels A, B, and C show confocal images of cryosections of a kidney-biopsy specimen that has been double-labeled with rabbit polyclonal anti–bovine serum albumin (anti-BSA) antibodies (Panel A, green) and anti–human IgG antibodies (Panel B, red). Panel C shows the merged image. Anti-BSA antibodies were preincubated with BSA, shown in Panel D; staining was eliminated despite the continued presence of IgG, as shown in Panel E. Panel F shows a biopsy specimen incubated with anti–human serum albumin (anti-HSA) antibodies, which was negative. Panel G shows immunostaining of a biopsy specimen with mouse monoclonal anti–human C5b–C9 antibodies followed by goat anti–mouse IgG antibodies. Panel H shows the reactivity of eluted IgG from a patient with (first and third panels) and a patient without (second panel) BSA deposits. The eluted IgG was used to immunoblot BSA and HSA. Only IgG4 and IgG1 eluted from the biopsy specimen with BSA in glomeruli detected BSA, whereas they did not detect HSA; staining of this biopsy specimen is shown in Panel I.

tigen implantation and subsequent in situ formation of immune complexes.[16-18]

Human exposure to bovine serum albumin is common through the diet and may also occur as part of medical therapy.[13] In young children, cow's milk is a major source of bovine serum albumin. Small amounts of dietary proteins may be absorbed in an undigested or partially digested form from the gastrointestinal tract in healthy persons.[20,21] IgG antibodies to cow's milk proteins are present in virtually all infants exposed to cow's milk and have been considered physiologic.[22] Although circulating antibodies to bovine serum albumin have been detected in many human serum specimens, they were not associated with any detectable clinical event,[13] except for IgE-mediated cow's milk allergy.[23]

In our patients with membranous nephropathy, most anti–bovine serum albumin antibodies were directed against a peptide of bovine serum albumin that comprises amino acid residues 147 to 161. Data on genetic susceptibility to this peptide are lacking. Anti–bovine serum albumin antibodies directed to other regions of bovine serum albumin have been identified in rheumatoid arthritis and multiple sclerosis, where they cross-react with collagen and myelin basic protein, respectively.[14,15] However, we could not identify sequence homologies between bovine serum albumin 147-161 peptide and podocyte proteins with computational sequence alignment. Moreover, antibodies from patients with membranous nephropathy that were affinity-purified against the bovine serum albumin 147-161 peptide did not recognize the proteins isolated from human glomeruli in Western blot analysis (data not shown).

Taken together, these observations suggest that at least two additional factors are necessary for membranous nephropathy to develop: the first is the physicochemical properties and amount of circulating antigen; the second is the predominance of the T-helper type 2 (Th2) immune response.

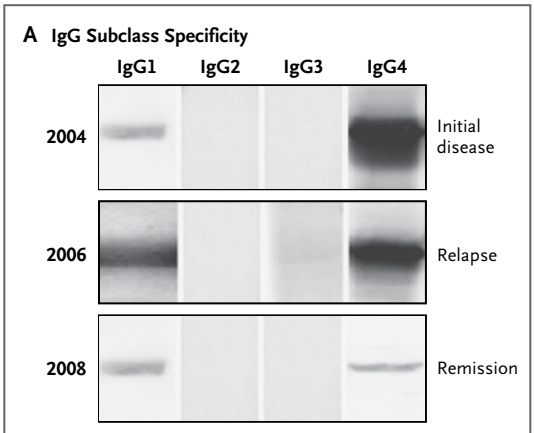
In bovine serum albumin–induced experimental models of membranous nephropathy, charge of the antigenic protein is a key factor for disease induction. Membranous nephropathy developed only in the animals that were given chemically modified cationized bovine serum albumin intravenously after immunization with cationic bovine serum albumin.[16-18] When native, slightly acidic bovine serum albumin was injected, the disease did not develop in the animals. Similarly, we found that bovine serum albumin could specifically be detected in immune deposits only in the patients who had both circulating cationic bovine serum albumin and bovine serum albumin–specific antibodies. The cause of the formation of cationic bovine serum albumin in our patients remains obscure. Further research is required to explain the differences in the technological processing of food and local intestinal microbiota that lead to pathologic modifications of bovine serum albumin in children.

Heat treatment of bovine serum albumin denatures the protein and results in reduced prote-

olysis[24] in the relatively high pH (3 or 4) of infants' stomach as compared with that of adults (pH 2).[25] Furthermore, the amount of intact bovine serum albumin entering the circulation is probably higher during infancy, before the gastrointestinal tract has matured and its barrier function has been established.[26,27] This amount may be increased during childhood gastroenteritis.[28] Both the predominance of a cationic form and the increased amount of absorbed bovine serum albumin most likely contribute to the development of membranous nephropathy in young children.

Membranous nephropathy is characterized by a predominant Th2 immune response with production of IgG4 both in humans and in animals.[29,30] Similarly, our patients with bovine serum albumin–induced membranous nephropathy had mainly IgG4 accompanied by IgG1 antibodies. IgG4 is unique among the IgG subclasses because it weakly activates complement and behaves mainly as a monovalent immunoglobulin.[31-33] Therefore, IgG4 can form small, nonprecipitating immune complexes that escape clearance and are difficult to detect. Although direct interaction of cationic bovine serum albumin with the anionic glomerular capillary wall most likely is the triggering event, one can speculate that circulating IgG4-containing immune complexes may subsequently be involved because of their longer half-life and their possible dissociation at the glomerular endothelium site, owing to the usual low affinity of IgG4 for antigens.[34] Because of the limited volume of available serum samples from the young children in our study, we could not search for IgG4-containing immune complexes by means of ultracentrifugation and size-exclusion chromatography. However, it is likely that small amounts of anti–bovine serum albumin IgG1 play an important pathogenic role, as previously shown in neutral endopeptidase–induced alloimmune neonatal membranous nephropathy.[4]

The identification of an environmental trigger as a causative antigen in early-childhood membranous nephropathy has important diagnostic and therapeutic consequences. Idiopathic membranous nephropathy is rare in children, accounting for the findings in approximately 2% of children who undergo renal biopsy, but it can be challenging to treat.[2,35] Although further epidemiologic studies are needed, absorption of dietary modified bovine serum albumin and immuniza-



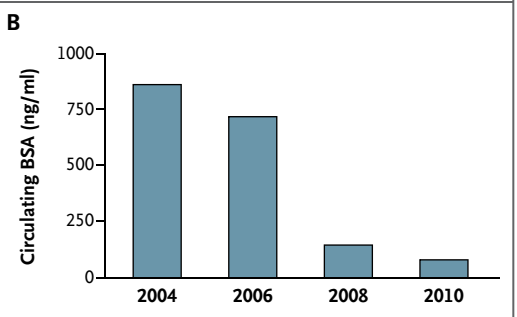


Figure 5. Anti–Bovine Serum Albumin Antibodies, Circulating Bovine Serum Albumin (BSA), and Disease Activity in a Patient with Membranous Nephropathy.

Serum samples were obtained from a boy who was 2 years 3 months of age. In June 2004, he received a diagnosis of membranous nephropathy (stage I) that caused severe nephrotic syndrome (urinary protein level, 9.45 g per 24 hours; serum albumin level, 21.8 g per liter; and serum creatinine level, 30 μmol per liter). He was treated for 4.5 months with prednisone, which was started at a dose of 60 mg per square meter of body-surface area per day for 1 month and then tapered to 60 mg per square meter every other day for 2 months and progressively discontinued over a period of 6 weeks, with resolution of proteinuria within 3 months. At the age of 4 years 6 months (in November 2006), a relapse of the initial disease occurred (urinary protein, level 10.1 g per 24 hours; serum albumin level, 25.6 g per liter; and serum creatinine level, 18 μmol per liter). Kidney-biopsy specimens showed stage I–II membranous nephropathy. He was treated with the same prednisone treatment protocol as initially, with complete normalization of all serum values and disappearance of proteinuria. In December 2008, the urinary protein level was in the normal range, with normal renal function. Complete remission persisted at the last follow-up, when the child was 8 years of age. Panel A shows the IgG-subclass specificity to BSA in serum samples analyzed by means of Western blot. Panel B shows the amount of circulating BSA.

tion against bovine serum albumin should be considered a potential cause of membranous nephropathy in young children and should prompt a search for bovine serum albumin in immune deposits. If bovine serum albumin is detected, eliminating it from the diet could be beneficial. More generally, our findings raise the possibility that other food antigens might be involved in the development of membranous nephropathy.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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