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Bovine serum albumin detected in infant formula is a possible trigger for insulin-dependent diabetes mellitus

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A constituent of cow's milk, bovine serum albumin (BSA), has recently been implicated as a possible trigger of insulin-dependent diabetes mellitus (IDDM) (1). Levels of serum antibodies to BSA were significantly elevated in children newly diagnosed with diabetes compared with normal control subjects (1,2), and cow's milk protein was diabetogenic in animal models of IDDM (3,4). In the latter studies, rats fed cow's milk protein at the onset of weaning (day 13) had an incidence of diabetes 2.6-fold greater than animals receiving milk-free protein during weaning and milk protein after weaning (day 23) (4). A Finnish study demonstrated that children who were older than 4 months of age when supplementary feeding (food or formula) was started had a lower risk of IDDM than those whose diets were supplemented before this age (5). Hence, there may be a window of time in early infancy when the consumption of cow's milk protein raises the risk for later development of IDDM.

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Some researchers think that destruction of pancreatic β-cells could result from an immune response to similar regions (ie, antigens) shared by BSA and human pancreatic β-cells (1). A 17-amino-acid region of BSA, termed ABBOS, is homologous with a surface protein on human pancreatic β-cells (6); hence, an immune response directed at BSA could react with self-antigens, leading to IDDM. In addition, genetic factors (7-9) and viral infections (10) influence susceptibility to autoimmune disease by influencing antigen availability, antigen presentation, and antigen recognition, and they are strongly implicated in autoimmunity.

If the genetic basis and accompanying environmental factors are appropriate, consumption of cow's milk protein during infancy may trigger the later development of IDDM (9). Cow's milk–based infant formulas are consumed by a majority of US infants. All major brands of commercially available milk-based formulas list as their first ingredient, aside from water in liquid products, "reduced minerals whey" or nonfat milk, both of which contain whey protein. Given the knowledge that BSA is present in the whey fraction of dairy products, we tested nine popular infant formulas, 2% cow's milk, and pooled human milk for BSA.

METHODS

Top-selling commercial infant formulas (four powdered and five liquid) and 2% cow's milk were purchased from supermarkets in the Phoenix, Ariz, area. All of the powdered formulas were based on cow's milk; three were low in iron and the fourth, which contained enzymatically hydrolyzed whey, was fortified with iron. Four of the liquid formulas were milk based; two were low in iron and two were fortified with iron. The remaining liquid formula was soy based and iron fortified. Human milk samples were collected from seven women twice weekly for 8 weeks. For each collection, women were instructed to pool a small sample of milk from each feeding over a 24-hour period, approximately 50 mL/day. These collections were pooled, freeze dried, and stored in glass under nitrogen at –2°C. Lyophilized human milk was reconstituted to original concentration of solids, and powdered infant formulas were diluted as specified by label directions.

All samples to be tested were ultracentrifuged (4°C) to remove fat and casein micelles (39,000 revolutions per minute, radius=6 inches, 18 hours), and the supernatant (undiluted) was analyzed by radial immunodiffusion (11). Two percent agarose was dissolved in veronal buffer (2 g/100 mL; pH 8.6) in a boiling water bath and allowed to equilibrate to 52°C. Equal volumes of rabbit anti–bovine serum albumin

(Sigma Chemical Co, St Louis, Mo; 1:50 in veronal buffer) and agarose were mixed at 52°C. Aliquots of the warm mixture (5 mL) were layered onto microscope slides, and 4-mm wells were cut once the antibody-containing agarose had hardened. A 5 µL aliquot of samples or BSA standard was pipetted into separate wells and incubated in a moist chamber for 48 hours at 4°C. Plates were washed in 1*M* sodium chloride (2 x 12 hours, 4°C), washed in deionized water (4 hours at 4°C), and dried (37°C) before staining with Coomassie Blue (Sigma B-0770, St Louis, Mo). All samples were tested in triplicate, and BSA was quantified by measuring the diameter of the precipitate ring.

RESULTS AND DISCUSSION

Three of the four powdered infant formulas and cow's milk tested positive for BSA; all five of the liquid formulas and human milk tested negative for BSA. The three powdered formulas with BSA contained 0.47 to 1.14 µmol BSA per liter (31.2 to 75.5 µg BSA per milliliter) compared with 2% cow's milk, which contained 1.14 µmol BSA per liter (75.5 µg BSA per milliliter) (Table). However, because protein concentrations in formula are low compared with cow's milk (1.4% vs 3.3%), BSA concentration in infant formula as a percentage of total protein is equal to, or in the case of one formula, almost twofold greater than that of cow's milk (Table). The single powdered formula that tested BSA-free contained whey as the primary ingredient; however, unlike the other powdered formulas, the whey was "enzymatically hydrolyzed," a process that may predigest the protein into peptides that are not immunogenic.

We were concerned that the heat of sterilization required by the retorted liquid product or the hydrolyzation process might denature the peptides to the degree that they would not migrate through the agarose gel. Hence, if BSA was present in the formula but could not migrate, our method for detecting BSA would not be adequate, because radial immunodiffusion only measures migrating proteins.

To examine this question, we prepared antibody-free agarose gels and allowed proteins from all samples tested to migrate into the gels for 30 minutes. The gels were then rinsed, dried, and stained for protein. The powdered formula that tested BSA-free did not display any migrating protein, whereas the three powdered formulas containing BSA and all of the liquid formulas displayed a band of migrating protein around the well. Our methodology, therefore, cannot determine whether the powdered formula that tested BSA-free by radial immunodiffusion is truly free of BSA, because no protein migration was observed. However, all of the liquid formulas tested BSA-free by radial immunodiffusion and demonstrated protein migration on antibody-free agarose; hence, these preparations do appear to be BSA-free. Liquid formulas are generally heat treated (240°F) for preservation, and apparently this procedure destroys the immunogenicity of BSA. (During the pasteurization process, milk is heated only to 185°F and BSA is not denatured.)

Although some of the formulas tested BSA-free, the radial immunodiffusion procedure is not sensitive enough to determine whether the BSA subunit, ABBOS, is present; hence, the BSA-free formulas may still possibly contain ABBOS. More sensitive assay procedures (eg, the enzyme-linked immunosorbent assay) will be necessary to demonstrate that formulas, or even human milk samples, are free of ABBOS.

If BSA is a trigger for IDDM, the presence of BSA in some infant formulas is particularly distressing, because formulas are consumed at a point in life when the digestive tract permits large protein chains to pass directly into the bloodstream. Encouragingly, our data indicate that perhaps certain processes may be adapted by the manufacturer during formula preparation to solve this dilemma.

Human milk did not test positive for BSA and, interestingly, infants breast-fed exclusively are at a lower risk for IDDM (12).

APPLICATIONS

Not every baby who drinks cow's milk or cow's milk–based infant formulas will develop IDDM. Genes have been identified that predispose individuals to the development of autoimmune diseases; furthermore, the timing of exposure to BSA and the timing and number of viral infections in early childhood are all implicated in the development of IDDM. Infants with a high risk of developing IDDM, however, should be breast-fed or given soy-based infant formula. Furthermore, manufacturers of infant formulas should be encouraged to develop processing procedures to destroy the BSA and its ABBOS subunit. Of course, breast milk is best, and the revised recommendations of the American Academy of Pediatrics state that infants should be breast-fed for the first 6 to 12 months of life and that whole cow's milk should be avoided for the first year of life (13).

Table
Bovine serum albumin (BSA) content of commercially available powdered infant formulas based on cow's milk (formulas 1-4), 2% cow's milk, and pooled human serum

Formula	µmol/L[a]	% total protein
Powdered formulas[b]		
1 = low iron	1.14	0.51
2 = hydrolyzed, high iron	0.00	0.00
3 = low iron	0.47	0.21
4 = low iron	0.52	0.24
2% cow's milk	1.14	0.23
Pooled human milk[c]	0.00	0.00

[a]To convert µmol/L BSA to µg/mL, divide µmol/L by 0.0151.
[b]The five liquid formulas examined did not test positive for BSA.
[c]Random aliquots of milk were collected from seven women twice weekly for 8 weeks.

References

1. Karjalainen J, Martin JM, Knip M, Ilonen J, Robinson BH, Savilahti E, Akerblom HD, Dosch HM. A bovine albumin peptide as a possible trigger of insulin-dependent diabetes mellitus. *N Engl J Med.* 1992; 327:302-307.

2. Savilahti E, Akerblom HK, Tainio VM, Koskimies S. Children with newly diagnosed insulin-dependent diabetes mellitus have increased levels of cow's milk antibodies. *Diabetes Res.* 1888; 7:137-140.

3. Elliott RB, Martin JM. Dietary protein: a trigger of insulin-dependent diabetes in the BB rat? *Diabetologia.* 1984; 26:297-299.

4. Daneman D, Fishman L, Clarson C, Martin JM. Dietary triggers of insulin-dependent diabetes in the BB rat. *Diabetes Res.* 1987; 5:93-97.

5. Virtanen SM, Rasanen L, Aro A. Infant feeding in Finnish children <7 years of age with newly diagnosed IDDM. *Diabetes Care.* 1991; 14:415-417.

6. Glerum M, Robinson BH, Martin JM. Could bovine serum albumin be the initiating antigen ultimately responsible for the development of insulin dependent diabetes mellitus? *Diabetes Res.* 1989; 10:103-107.

7. Carson DA. Genetic factors in the etiology and pathogenesis of autoimmunity. *FASEB J.* 1992; 6:2800-2805.

8. Robinson BH, Dosch HM, Martin JM, Akerblom HK, Savilahati E, Knip M, Ilonen J. A model for the involvement of MHC class II

proteins in the development of Type 1 (insulin-dependent) diabetes mellitus in response to bovine serum albumin peptides. *Diabetologia.* 1993; 36:364-368.

9. Kostraba J, Cruickshanks RJ, Laeier-Heavner J, Jobim LF, Rewers MJ. Early exposure to cow's milk and solid foods in infancy, genetic predisposition, and risk of IDDM. *Diabetes.* 1993; 42:288-295.

10. Barnett LA, Fujinami RS. Molecular mimicry: a mechanism for autoimmune injury. *FASEB J.* 1992; 6:840-844.

11. Mancini G, Carbonara AO, Heremans JF. Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry.* 1965; 2:235-254.

12. Mayer EJ, Hamman RF, Gay EC, Lezotte DC, Savitz DA, Klingensmith GJ. Reduced risk of IDDM among breast-fed children. *Diabetes.* 1988; 37:1625-1632.

13. Committee on Nutrition, American Academy of Pediatrics. The use of whole cow's milk in infancy. *Pediatrics.* 1992; 89:1105-1109.