Review

Bovine serum albumin and insulin-dependent diabetes mellitus: is cow’s milk still a possible toxicological causative agent of diabetes?

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Abstract

The implication of bovine serum albumin (BSA) in cow’s milk as a causative agent for the onset of insulin-dependent diabetes mellitus (IDDM) is a major topic of scientific debate notwithstanding the medical and economic implications. A critical survey of the pertinent literature has revealed a number of controversies. For example, an important toxicological aspect of BSA is the presence of ABBOS, a peptide segment of the protein. However, the nature and effect of ABBOS on the death of insulin producing cells (β-cells of the pancreas) is unclear and hence inconclusive. In addition, studies in diabetes-prone mice and rats appear to show that cow’s milk does not alter the frequency of diabetes in these organisms. It is suggested that BSA may not be the cause of diabetes. Instead, IDDM is most likely the result of oxidative stress, due to high local levels of nitric oxide (NO•) and oxygen radicals (O2•−), on the β-cells of the pancreas, which eventually leads to their destruction.

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1. Introduction

Diabetes mellitus is a disease characterized by abnormal amounts of glucose in the blood and urine of patients having the disease. There are three main types of diabetes, insulin-dependent (IDDM or Type I diabetes), non-insulin-dependent (NIDDM or Type II diabetes) and gestational diabetes mellitus. Insulin-dependent diabetes mellitus most often develops in children and young adults. In the U.S.A., about 5–10% of the people with diabetes have IDDM. Non-insulin-dependent is the most prevalent form of diabetes (90–95%) and is common in adults over 40 years old who are usually overweight (NIH, 1994). Gestational diabetes develops in women during pregnancy. After the baby is born, this type of diabetes ends. However, there are cases where women who previously had gestational diabetes developed NIDDM as they aged (NIH, 1994).

In patients with IDDM, the pancreas produces either too little or no insulin at all. Insulin is a protein hormone (5.8 kDa) which is important in fuel metabolism and it is secreted by the β-cells of the pancreas. Insulin stimulates glycogen synthesis in both muscle and liver, and it suppresses gluconeogenesis. In other words, insulin facilitates the entry of glucose into muscle and adipose cells, and hence, it is important in removing glucose from the blood. Insulin is also important in amino acid and protein metabolism (Stryer, 1988).

In IDDM patients, the insulin producing β-cells in the pancreatic islets have been destroyed by the body’s immune system. Therefore, IDDM is an example of an autoimmune disease and as well, an organ-specific disease as the immune response is primarily directed towards antigens in selected organs. The symptoms of IDDM include, in addition to high blood glucose level, frequent urination, increased thirst, unexplained weight loss and extreme tiredness. In severe IDDM a condition known as ketoacidosis may develop which is the build up of ketones as a result of the catabolism of fats. The quest for a cure for IDDM has led scientists to determine the possible factors that trigger the onset of the disease. One of these factors that has been given considerable attention and as well stirred up controversies, is the implication of bovine serum albumin as a possible causative agent of IDDM.

In this paper, only IDDM will be discussed with respect to its possible cause by bovine serum albumin (BSA), a protein present in cow’s milk. The paper presents a critical review of the current literature and identifies weak points in the BSA hypothesis. In addition, this review discusses the possibility that oxidative stress is a major cause for the destruction of the β-cells and that the increased antibodies against BSA is the result of the body’s normal immune response to foreign proteins.

2. Bovine serum albumin and IDDM

2.1. Cow’s milk and the incidence of IDDM

It is generally accepted that IDDM is the result of a genetically transmitted autoimmune process that destroys the β-cells in the pancreatic islets of Langerhans. However, in studies performed on identical twins, it was found that the presence of IDDM was less than 50% (Olmos et al., 1988). This suggested that the disease, in part, was caused by non-genetic determinants (Kaprio et al., 1993; Verge et al., 1994). Of the possible non-genetic determinants, environmental factors, such as response to pathogens and dietary intake, have been implicated in triggering the autoimmune process (Verge et al., 1994; Guberski et al., 1991; Elliott and Martin, 1984). In animals that are diabetes-prone (e.g., Bio-Breeding [BB] rats and non-obese diabetic [NOD] mice, where the disease is primarily directed against the pancreatic β-cells) the animals had a greater chance of developing an autoimmune Type I diabetes, when fed from weaning on a diet of cow’s milk proteins. Exclusion of cow’s milk from the animals’ diet resulted in a lower incidence of the disease. These experiments therefore showed the influence of a diet based on cow’s milk proteins on the onset of diabetes Type I in animal models.

Epidemiological studies in human populations also suggest a similar relationship between the consumption of cow’s milk and Type I diabetes (Fig. 1) (Dahl-Jorgensen et al., 1991). Fig. 1 shows that countries with a large utilization of cow’s milk have a higher proportion of patients with IDDM diabetes in the age group 0–14 years. In other human studies, performed on children at risk for diabetes, it was found that those children who were exclusively breast-fed during the first few months of life were less likely to develop diabetes compared with at risk children who were fed on a diet of cow’s

Fig. 1. The relationship between the incidence of insulin-dependent diabetes mellitus in children 0–14 years of age by average fluid cow’s milk consumption per person per year in different countries. (Adapted from Dahl-Jorgensen et al., 1991).
milk formula (Verge et al., 1994; Rennie, 1992; Virtanen et al., 1993; Kostraba et al., 1993; Borch-Johnson et al., 1984).

It is believed that the protein, BSA, in cow’s milk, is a possible agent that triggers the autoimmune response which leads to IDDM. The implication of BSA was based on the presence of increased levels of antibodies directed against BSA especially that of IgG anti-BSA antibodies in the research carried out by Karjalainen et al. (1992) and later by Hilger et al. (2001). The significance of their work was that it was the first report that showed a very strong IgG antibody reaction directed against a particular antigen. This is important because any major target for antibodies is most likely going to be implicated in the onset of the disease.

Bovine serum albumin is a 69 kDa protein present in cow’s milk (0.1–0.4 g L⁻¹) and it is identical (i.e., similar but not exact homology) to human blood serum albumin (Eigel et al., 1984; Morr and Ha, 1993). The primary amino acid sequence of BSA is shown in Fig. 2. The events leading to β-cell destruction, resulting from BSA, are thought to originate in early infancy, before the gastrointestinal tract has matured and its barrier function has been established (Karjalainen et al., 1992; American Academy of Pediatrics, 1992).

2.2. A hypothesis for the cause of IDDM by BSA in cow’s milk

Experimental studies have shown that most of the anti-BSA antibodies appeared to be directed against a section of the milk protein molecule that comprises amino acid residues 152–168 which is a region of dissimilarity between human and bovine serum albumins (Fig. 2). This amino acid region is also known as the ABBOS section and it is immunogenic in hosts that possess diabetogenic genotypes, HLA or MHC (DR/DQ) genes on human chromosome 6, that is able to bind and identify this antigenic peptide (Todd, 1990; Nepron, 1990; Robinson et al., 1993). Genetic susceptibility to type I diabetes is determined by the structure of the α and β-chains of DQ and the β-chain of DR (Todd, 1990; Robinson et al., 1993). However, IDDM occurs almost exclusively in genetically susceptible individuals who carry these specific alterations in single amino acids which involve position 57 on the DQ β-chain and position 52 of the α-chain (Karjalainen et al., 1994).

Partial digestion of BSA from cow’s milk by proteolytic enzymes in the immature gut results in the release of peptide fragments which later become absorbed. One of these peptides (e.g., ABBOS) is believed to contain an antigenic epitope recognized by T-cells and an epitope recognized by B-cells (Baxter and Cooke, 1992; Watson et al., 1992). In order that the foreign peptide be recognized by the T-cell, it (the epitope) must be bound to a particular membrane protein (one encoded by the major histocompatibility complex (MHC) located on the surface of the cell) and then presented to the T-cell (Robinson et al., 1993; Baxter and Cooke, 1992; Watson et al., 1992).

The MHC is a chromosomal region in the genes of individuals. This large region (∼3000 kb) encodes three classes of transmembrane proteins: (a) class I proteins that present foreign epitopes to cytotoxic cells (CD8 T-cells), in this case the infected cell is presenting the

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Fig. 2. The primary amino acid sequence of bovine serum albumin (BSA). Also shown is the ABBOS epitope (region 152–168, in italics), which is believed to be important in triggering the autoimmune response in individuals with a genetic predisposition to IDDM (Adapted from Eigel et al., 1984).
antigen epitope, (b) class II proteins that are usually located on a macrophage which presents the epitopes to helper CD4 T-cells, which synthesize and secrete lymphokines that cause antibody-producing B-cells to divide and secrete antibodies and (c) class III proteins that are components of the complement cascade (Stryer, 1988).

The immune response directed against BSA, according to Karjalainen et al. (1992), can be explained as follows. A macrophage encounters an antigen and it presents the antigen via its MHC II proteins to a helper T-cell, which recognizes the complex of the MHC class II proteins and the antigenic peptide. The T-cells then secrete lymphokines that signal B-cells to differentiate into plasma cells, which synthesize and secrete antibodies that bind the BSA in the blood stream (Fig. 3) (Karjalainen et al., 1994; Baxter and Cookie, 1992).

Important in the T-cell receptor-MHC class II complex is the presence of a surface glycoprotein CD4 (55 kDa) which is associated with class II MHC-specific helper T-cells. These proteins are important in transmitting signals to the intracellular components which activate the T-cell. The CD4 protein is also the receptor through which human immunodeficiency virus (HIV), the virus that causes AIDS, enters the helper T-cells (Watson et al., 1992).

The islet β-cells produce a protein of 69 kDa (also known as p69) which comes to the surface of the cells to defend them against viral infections (Karjalainen et al., 1992). This p69 can be induced by the inflammatory mediator γ-interferon, a family of small proteins that is released from white blood cells in response to invading viruses (NIH, 1994; Karjalainen et al., 1992). This p69 protein is believed to possess a homology (epitope) of primary amino acid residues similar to the ABBOS peptide and hence the body triggers an immune response against the β-cells which leads to the destruction of these insulin-producing cells (Fig. 4). After the viral infection has subsided the p69 retreats inside the β-cells and destruction stops.

According to this “cow’s milk” theory for islet β-cells destruction, each time a child is infected by a virus the body kills the virus as well as a few more islet cells.

Eventually, when 90% of the β-cells have been destroyed, the child will begin to show signs of diabetes (Karjalainen et al., 1992). It is believed that cow’s milk introduced before 3 months of age is associated with increased risk of IDDM (Verge et al., 1994; Virtanen et al., 1993; Kostraba et al., 1993). At ages greater than 3 months, it is suggested that the immune system is able to distinguish between p69 and cow’s BSA. For example, epidemiological studies demonstrate that a cow’s milk diet introduced to babies 5–6 months old, showed reduced sensitivity by the babies to the milk protein (Verge et al., 1994; Kostraba et al., 1993).

3. Controversies

3.1. A critique of the cow’s milk theory

The studies on the effects of BSA in cow’s milk and its relation to the autoimmune disease of IDDM show a possible link between diet and the onset of diabetes. However, the implication is for children with a genetic predisposition to the disease. The theory of the antigen ABBOS in the production of diabetes still requires further clarification. For example, what would be the factor that cleaves this p69 on the surface of the β-cells? This is important as CD4 T-cell mediated destruction involves antigens that are presented by MHC class II proteins. But there is a discrepancy here as a direct attack on the β-cells (the so called ‘infected’ cell) would involve MHC Class I proteins (on the surface of the β-cells) and require a CD8 T-cell mediation (Watson et al., 1992). In addition, what is the purpose of p69 and its mechanism of activation against viral infection? And exactly how does the interferon create the induction of the surface presence of p69 on β-cells? To date, there has been no confirmation of the implications of either CD4 or CD8 T-cells or both in the destruction of β-cells. These questions need to be answered before a
strong influence of the ABBOS peptide of BSA is linked strongly to the onset of IDDM.

It must be noted that there are some inconsistencies in the data given for the amino acid sequence of ABBOS. For example, Karjalainen et al. (1992) gave a sequence for ABBOS as Ala-Asp-Glu-Lys-Lys-Phe-Leu-Gly-Lys-Tyr-Leu-Tyr-Glu-Ile-Ala-Arc-Arg-His, which is position 128–145 in the amino acid sequence of BSA (Fig. 2). A more recent paper by Robinson et al. (1993), gave a sequence for ABBOS as Ala-Asp-Glu-Lys-Lys-Phe-Leu-Gly-Lys-Tyr-Leu-Tyr-Glu-Ile-Ala-Arg-Arg-His, which is position 128–145 in the amino acid sequence of BSA (Fig. 2) and not 152–168. Also, Karjalainen et al. (1992) have implicated that trypsin would cleave the ABBOS peptide from BSA and this would leave most of the peptide epitope intact; this is possible for region 152–168 but not for region 128–145. Trypsin is a serine protease which specifically cleaves on the carboxyl side of Arg and Lys residues, since region 128–145 contains a larger number of these amino acid residues, the ABBOS peptide (if composed of residues 128–145 instead) would almost completely be broken down before entrance into the gastro-intestinal tract (GIT) of the infant.

The amino acid sequence of the human p69 protein has been determined (Pietropaolo et al., 1993; Miyazaki et al., 1994). Pietropaolo et al. (1993) and Miyazaki et al. (1994) have each identified two regions of similarity between human p69 and BSA (Pietropaolo et al., 1993; Miyazaki et al., 1994), however, by combining the result of these two papers there are actually three regions of similarity (Fig. 5). Interestingly, the region of similarity is not between the region 152–168 of BSA as suggested by Karjalainen et al. (1992). The similarities exist in the regions 127–132, 170–179, and 371–380. There have been no reports on the study of antibodies produced against these homologous regions as shown in Fig. 5, and clearly these regions should be studied if BSA is to be implicated in the onset of IDDM.

Important as well, are that these regions also show some similarity in homology to human serum albumin (Pietropaolo et al., 1993) based on the theory of cross reactivity and molecular mimicry by Karjalainen et al. (1992) there should be antibodies developed even against human serum albumin and this fact complicates the theory of the implication of BSA. The presence of these homologous regions brings to mind the following questions: (i) what genetic variants of BSA are being compared with p69? (ii) how much similarity must there be in the sequence of BSA and p69 to stimulate β-cells destruction? (iii) are the similarities as shown in Fig. 5 significant or just coincidence? and (iv) is the nature of the secondary and/or tertiary structure of these ‘homologous’ regions important in the recognition process or is the amino acid sequence more important?

The key fact in the support of the ‘cow’s milk’ theory is that there are increased levels of antibodies (Ig-G and Ig-A) and there were no significant increases in levels of antibodies against the other milk proteins present (Karjalainen et al., 1992). However, there have been reports that show that there are increased levels of Ig-A antibodies against β-lactoglobulin (β-lg), another protein present in cow’s milk (2–4 g L\(^{-1}\)), by Dahlquist et al. (1992) and Lorni et al. (1993). These authors suggest that even β-lg may be one of the triggers for the onset of Type I diabetes in genetically susceptible individuals (Dahlquist et al., 1992; Lorni et al., 1993).

Another report which does not support the cow’s milk theory was given by Atkinson et al. (1993), who based their work on the presence of mononuclear cell infiltration of the pancreatic islets. They suggested that cellular rather than serological immunity is more associated with IDDM. As a result, they analyzed the response of peripheral-blood mononuclear cells to BSA and ABBOS and the formation of IgG anti-BSA antibodies in patients with newly diagnosed IDDM. They found no significant amount of peripheral-blood mononuclear cells to BSA and ABBOS and also, no significant increase in concentrations of IgG anti-BSA antibodies. Hence, they questioned the relevance of ABBOS and BSA to the pathogenesis of IDDM. The work by Atkinson et al. (1993) also showed that anti-BSA antibodies were found in patients with other autoimmune diseases (e.g., systemic lupus erythematosus and rheumatoid arthritis) and the authors believed that there is probably no specificity for BSA but rather a general immune response against BSA. After all, there is even
the presence of antibodies against BSA in normal healthy patients (Karjalainen et al., 1992).

Bovine serum albumin, therefore, may just be one of the many stimuli that triggers a general immune response and may not be the cause of the autoimmune response. There are other proteins or other macromolecules that create such a general immune response, for example, gliadin and soybean products in the diets of IDDM patients (Elliott and Martin, 1984). The link between proteins and the onset of diabetes in BB rats and NOD mice stipulated such a relationship, however, there are studies which have not detected higher diabetes frequencies in BB rats and NOD mice that were fed diets of cow’s milk proteins (Scott et al., 1994; Malkani et al., 1997). In addition, a cow’s milk-free diet did not stop the presence of diabetes in NOD mice (Paxson et al., 1997). Therefore, a significant relationship between BSA antibodies and the later onset of diabetes cannot be made.

3.2. Type I diabetes, inflammatory or autoimmune?

Another controversial view is whether Type I diabetes is actually autoimmune or inflammatory. That is, Type I diabetes is possibly due to the selective death of β-cells as a result of a non-specific inflammatory attack (Kolb et al., 1995). In other words, diabetes can develop in the absence of MHC class I or class II restricted cytotoxicity and the destruction of β-cells is the result of indirect killing via activated CD4 or CD8 T-cells. It is suggested that the death of β-cells is the result of high local levels of nitric oxide (NO), oxygen radicals (O₂) and certain cytokines, which are produced as a result of an inflammatory response mediated by CD4 and CD8 cells (Karjalainen et al., 1994; Kolb et al., 1995; Atkinson and Maclanen, 1994). It is suggested that of all the islet cell types, β-cells have the least resistance to these toxic mediators released during islet inflammation (Kolb et al., 1995). It is believed that the triggering of this inflammatory attack may be the result of the production of an autoantigen (s), e.g., glutamic acid decarboxylase (GAD), insulin, heat shock protein 60, a 38 kDa protein and p69 in IDDM patients (Bach, 1995). The mechanism by which these autoantigens develop an immune response is still unknown. However, a single autoantigen may give rise to an initial attack to the specific target organ which stimulates a T-cell mediated inflammation (Fig. 6).

As a result of β-cell destruction there may be the release of its immunogenic cell components that could

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Fig. 6. (a). The inflammatory theory of β-cell destruction. A β-cell produces an autoantigen (s). The autoantigen (p69 or GAD?) binds to MHC Class I or II antigen producing cells (e.g., macrophages) and not to the MHC Class I or Class II proteins on the β-cell itself. (b) The bound autoantigen is then presented to the cytotoxic T-cell (CD4 or CD8) for destruction (Primary immune response). This inflammatory response results in the production of nitric oxide (NO) and oxygen radicals (e.g., O₂⁻) which then destroy the sensitive β-cells.
lead to a secondary autoimmune response which could be difficult to distinguish from the previous one. Fig. 6 shows the general immune response against BSA and the similarity between the autoantigen p69 and BSA epitopes results in an increase in antibodies directed against p69 (a possible secondary autoimmune response?). Support of the latter hypothesis came when it had been shown in NOD mice that GAD is probably the first macromolecule to be produced in the onset of diabetes (the first sign of autoimmunity) (Atkinson and Maclanen, 1994; Eisenbarth, 1994) and hence the role of BSA in initiating an autoimmune response has been questioned again.

3.3. Applicability of animal models

Another controversial issue is the applicability of animal models to the case of human diabetes. For example, doses of substances administered to animals are significantly higher than that which would be obtained in a normal diet by humans. In addition, animal species are known to react differently to the same toxins or antigens, as one species may experience toxicity and another may not. Also, is it possible that experimental manipulation of animals can create diabetes as a result of other immune mechanisms?

3.4. Significance of epidemiological studies

Epidemiological studies on the incidence of IDDM in children in various countries have shown a possible link between cow’s milk and the incidence of IDDM. However, such data must be taken with caution as it does not take into consideration a number of confounding variables. For example, Fig. 1 is based on the consumption of milk (l/person/year) in each country. Do these data reflect cow’s milk as the diet of those prone to diabetes or the whole population? In addition, the epidemiological data do not consider cultural background, the influence of other food constituents in the diet of the individuals, the influence of the mother’s diet, effect of the mother’s education, and time of introduction of the child to daycare (Verge et al., 1994). The epidemiological study (Fig. 1) also shows a gradient in the consumption of cow’s milk from countries in the North to the South. Note that Fig. 1 does not consider the fact that the diet of the population is not solely milk, for example, the northern hemisphere consumes a large percentage of coffee and it is possible that a similar relationship could exist based on the mothers consumption of coffee (yet an unlikely, and unrelated link to IDDM). For the epidemiological study to be more conclusive it would need to compare patients fed on cow’s milk with otherwise identical patients fed on mother’s milk. Confounding variables like parents income, imports vs. exports, treatment and processing of milk and the diet (type of feed) of cows would also have to be taken into account.

3.5. Appropriate introduction of cow’s milk in infant diets

The possible role of BSA as a potential trigger of IDDM has revived previous controversies about cow’s milk and the optimal feeding habits for infants. A total elimination of cow’s milk from the diet may not be the complete answer as some babies that were solely breast fed for the first few months still developed the disease (Verge et al., 1994; Borch-Johnson et al., 1984). Cow’s milk has low iron concentration, which could lead to iron-deficiency anemia; other problems are colic, food allergies and atherosclerosis. Relevant to allergic reactions to milk proteins is the controversy as to when is the best time for the introduction of cow’s milk in an infant’s diet. In newborns, the GIT is not matured and is permeable to foreign macromolecules, which can lead to increased sensitivity and the onset of allergic reactions (American Academy of Pediatrics, 1992). Nevertheless, it has been suggested by the American Academy of Pediatrics that infants should be fed cow’s milk products and cow milk formula (fortified with iron and vitamins) after 6 months of age when it is believed that the GIT barrier is fully matured. However, it is difficult to exclude cow’s milk proteins as many products are made from them e.g., food emulsions, colloids etc., and the effect of the mother’s diet, if it includes dairy products, on the growing infants is not known.

4. Conclusions

This paper presented information from the literature which suggests bovine serum albumin may be implicated in the onset of IDDM. It also reported the epidemiological studies performed and identified the caution needed when interpreting such information. The implication of cow’s milk in the diet of infants as a possible cause of IDDM has stirred considerable controversy. Overall, the information from the literature shows that the increased antibodies produced against BSA may just be the result of the body’s normal immune response to foreign proteins and the death of the β-cells may be due to their sensitivity to oxidative stress. Some facts still need to be clarified about BSA, for example, what is the exact location and amino acid sequence of the BSA epitopes, and what genetic variants of BSA are being used in the analysis? Follow-up on anti-BSA antibodies and peripheral-blood mononuclear cells directed against BSA and ABBOS needs to be carried out in other laboratories to validate and further enhance our understanding of the implications of BSA with respect to IDDM. Any major implication of cow’s milk in the
onset of IDDM needs to be established without any considerable reason for doubt, as elimination of cow’s milk (and at what age this should occur) would have a serious economic effect and a tremendous impact on the feeding of infants, especially if cow’s milk is a major dietary food source.

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