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Relation of Time of Introduction of Cow Milk Protein to an Infant and Risk of Type-1 Diabetes Mellitus

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Several studies of infant feeding show a causal relationship between time of introduction of formula containing cow protein and risk of onset of type-1 diabetes mellitus. This paper cites the literature pro and con and discusses lipocalins which might play a role in the pathogensis. β Lactoglobulin, a major lipocalin protein in bovine milk, is homologous to the human protein glycodelin (PP14), a T cell modulator. Anti-β lactoglobulin cross-reacts with glycodelin. The newborn intestine does not have complete "closure" and can pass food antigens. β Lactoglobulin could generate antibody to glycodelin undermining T cell regulation of beta cells.

Keywords: β lactoglobulin • glycodelin • type-1 diabetes mellitus

The intent of this communication is to review the relationship between lipocalins β lactoglobulin, a major whey protein of bovine milk, and a human protein of pregnancy, glycodelin (PP14), an immunomodulator, and the possible negative effect on insulin producing beta cells. Lipocalins are known allergens,[1] and β lactoglobulin has been identified as a cow's milk allergen. [2,3] Bovine milk plays a large role in the human diet, particularly with young children. If a newborn is not breastfed, formula, a modification of cow's milk, is the sole source of nutrition. The possibility of antigenic cross reactivity with the protein glycodelin which could influence autoimmune activity needs to be investigated. Type-1 diabetes mellitus is an autoimmune disease.

The literature is equivocal about the relationship of cow milk products, particularly the relationship between time of introduction to an infant and the onset of type-1 diabetes mellitus. A large controlled case study in Finland[4] concluded that the age of introduction of dairy products is associated with risk of type-1 diabetes mellitus. A review paper "Milk and Diabetes"[5] lists several studies which suggest cow milk products are associated with the onset of type-1 diabetes and several other studies which show no correlation. In addition, the work of Fort et al.[6] and Wasmuth et al.[7] does not support the conclusion, while Cavallo et al.,[8] Saukkonen et al.,[9] and, recently, Rosenbauer et al.[10] do. These studies involve analysis of exclusive breastfeeding and time of introduction of formula. The supporting studies found introduction of formula before 4 months of age had a significant relationship with disease onset.

The Finland case study (matched samples and controlled for multiple factors)[4] looked at 690 type-1 diabetes mellitus children <14 years, diagnosed between September 1986 and April 1989. The study was set up to distinguish between effect of duration of breast feeding and time of introduction of dairy products. The study found increased risk with early introduction of dairy products. The study cites Savilahti et al.[11] who found increased levels of IgA and IgG antibody to β lactoglobulin in newly diagnosed diabetic children compared with nondiabetic. Virtanen et al. later confirmed patients <3 years had markedly higher IgG and IgA to cow milk and IgG antibodies to β lactoglobulin.[12] IgA antibodies to β lactoglobulin were shown to associate with risk for type-1 diabetes.[13]

Lipocalins are a large group of small extracellular proteins with diversity at the sequence level, but highly conserved crystal structures.[14] They have the ability to bind a range of small hydrophobic molecules.[15] A subset, $\alpha_1$acid glycoprotein, $\alpha_1$microglobulin. and glycodelin, which exert significant immunomodulatory effects in vitro has been termed immunocalins.[16] Glycodelin has three well-defined isoforms,[17] glycodelin-A, glycodelin-S, and glycodelin-F. All have the same protein sequence, but different carbohydrate moieties. This suggests the glycans mediate the biological actions.[18] Glycodelin-A, synthesized in the endometrium, is abundant in amniotic fluid. Glycodelin-S is abundant in seminal plasma. Glycodelin-F is found in follicular fluid.[19]

β Lactoglobulin is present in the milk of ruminants, but not humans.[20] It has the largest concentration of any whey protein in bovine milk, 3 g/L. A 1986 paper in Nature[21] suggested the structure was similar to plasma retinol-binding protein, and its function might be vitamin A transport. It has an unusual protein fold composed of two slabs of antiparallel β sheets. A reversible change can occur aound pH 7.0 known as the Tanford transition causing a change in conformation.[22] The function of the protein has never been confirmed, but it is known to bind many hydrophobic ligands. The common genetic variants are A and B. Cows are described as phenotype A/A, B/B, or A/B.[23] Isoform A shows a slightly lower molecular size on SDS-PAGE.[2] β Lactoglobulin exists as a monomer–dimer mixture in solution moderated by pH and salt concentration.[24] At its pI 5.2, it is a stable dimer of 34 kDa. Between pH 3.4 and

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5.2, dimers of both variants associate to form octamers with association stronger with β lactoglobulin A. Antibody raised to either variant recognizes both, but β lactoglobulin A generated a greater antibody response.[2]

Glycodelin has significant homology with β lactoglobulin.[25,26] It is a major soluable protein of pregnancy and is known to directly modulate T cells.[27,28] It is a 28 kDa glycoprotein particularly expressed in steroid-responsive tissues of the female reproductive tract; the endometrium in response to progesterone. There is a large concentration in amnionic fluid which is thought to be a nutritive protein for the fetus in early pregnancy.[29] It is a negative regulator in T cell receptor-mediated activation.[30] Foth et al.[31] show that endometrium and ovary are not the only source of the protein, and Morrow et al.[32] identify a hematopoietic glycodelin (PP14) with anti-PP14 antibody. Antibody raised to β lactoglobulin cross-reacts with glycodelin.[33]

Milk is species-specific. Between 2 and 3% of children under 2 years have cow's milk allergy, an IgE moderated disease.[3] It is speculated that most adults have some IgG antibodies to cow milk proteins which do not appear to cause any ill effects. Researchers[34] have looked at IgG and IgM antibody to cow's milk proteins in sera of type-1 diabetes mellitus individuals. IgG levels to casein were higher in type-1 diabetes children, but not significant. Also, serum IgG to bovine insulin was investigated.[35] Neither study identified antibody with a relationship to pathogenesis.

In our laboratory, we looked at 5 sera from individuals between 20 and 30 years, 2 males and 3 females, using immunoblots of 2D separations of cow's milk. All 5 had antibody to kappa casein, 4 had antibody to A_{s1} and β casein, 3 to butyrophilin, 2 to albumin, 1 to α lactalbumin; β lactoglobulin had 1 positive to the 34 kDa dimer and 17 kDa monomer doublet, and 1 positive just to the 17 kDa monomer doublet.

We looked at 5 sera from juveniles with type-1 diabetes: males 7, 10, and 13 years, and 2 females 14 years. Four had antibody to A_{s1}, A_{s2}, β and kappa casein. Four had antibody to butyrophilin, 3 to albumin, and 3 to α lactalbumin. All 5 had antibody to β lactoglobulin 34 kDa dimer, 17 kDa monomer doublet, and 16 kDa monomer doublet. None of the nondiabetic sera had antibody to the 16 kDa doublet. All β lactoglobulin spots were confirmed with A′β lactoglobulin antisera and by MS. β Lactoglobulin isoform A has a slightly lower molecular size. This suggests that the 16 kDa doublet is the A isoform. All type-1 positive sera had antibody to β lactoglobulin doublet spots at 16 kDa. None of the nondiabetic sera showed antibody to these 2 spots. Blast[25] did not identify any β lactoglobulin homology with islet-cell proteins, but significant homology to glycodelin.

Intestinal "closure" in humans (the loss of ability of the intestinal membrane to take up macromolecules) is thought to take place during the seventh–eighth month of fetal gestation.[36] However, Walker et al.[37] have shown that food antigens can still pass easily through neonatal intestine, and β lactoglobulin may generate antibody to glycodelin.

A complex situation may involve β lactoglobulin antibody eliminating glycodelin and allowing proliferation of autoreactive T cells. The immature immunogenotype of some infants may see the 16 kDa β lactoglobulin as foreign and produce antibody which cross-reacts with glycodelin. Type-1 diabetes mellitus is thought to be caused by autoimmune destruction of pancreatic β cells by T cells.[38] Destruction of glycodelin, a negative regulator, may allow proliferation of these cells. This intricate set of conditions may be the reason research to this point has not found a clear answer to the relationship of cow milk products and type 1-diabetes mellitus.

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