Cow's milk and immune-mediated diabetes

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Cow's milk-based infant formulas and cow's milk consumption in childhood have been suggested to promote the development of type 1 diabetes mellitus and other immune-mediated or neurological diseases. Epidemiological studies in man have led to the hypothesis that introduction of cow's milk-based infant formula within the first 3 months of life is associated with increased risk of type 1 diabetes mellitus. Furthermore, in animal models of type 1 diabetes mellitus, cow's milk proteins have been proven to be 'diabetogenic'. However, the issue seems far from being resolved. Several epidemiological studies and, more importantly, the first prospective trials did not show an association between early exposure to cow's milk and type 1 diabetes mellitus. In animal models, cow's milk proteins are modestly and variably diabetogenic, wheat or soyabean proteins in the diet cause higher rates of autoimmune diabetes. In both man and rodents there is increasing evidence that the gut-associated immune system plays a major role in disease development, probably because of disturbed oral tolerance mechanisms. Oral tolerance depends on immunological homeostasis and normal maturation of the gut. These factors are influenced by growth factors and cytokines from breast milk, normal bacterial colonization, infections and diet. All these factors have been proposed as risk factors for type 1 diabetes mellitus. Hence, cow's milk proteins may provide mimicry epitopes relevant in autoimmunity, as well as destabilizing oral tolerance mechanisms by biologically active peptides. The concept of dietary regulation of autoimmunity does not apply only to cow's milk protein, but also to other dietary proteins.

Cow's milk protein: Type 1-diabetes mellitus: Gut-associated immune system

Background for the hypothesis

In recent years there has been growing public concern that the early introduction of cow's milk products to infant diets might be a risk factor for the later development of immune-mediated (type 1) diabetes mellitus.

Evidence supporting this hypothesis mainly stems from epidemiological studies. About 15 years ago the first report was published which suggested an inverse association between the duration of breast-feeding and the incidence of autoimmune diabetes in Norway and Sweden (Borch-Johnsen *et al*. 1984). This observation was later extended by other studies demonstrating a positive correlation between the regional incidence of type 1 diabetes mellitus and the *per capita* consumption of cow's milk between (Scott 1990; Dahl-Jorgensen *et al*. 1991) or within countries (Fava *et al*. 1994). Based on these observations a still increasing number of case–control studies have been performed to evaluate the impact of cow's milk-based infant formula in the first 3–6 months on the later development of autoimmune diabetes (Kolb & Pozzilli, 1999). While approximately half these studies could demonstrate a link between the early introduction of cow's milk and type 1 diabetes mellitus the other half failed to do so (Scott *et al*. 1996). Two meta-analyses of the relevant studies showed a modest but significant increased risk for diabetes in children who were exposed to cow's milk before the age of 3 months (Gerstein, 1994; Norris & Scott, 1996). It is important to note that only children with a genetic predisposition would be at risk for type 1 diabetes mellitus. In two studies which included control groups with diabetes-associated human leucocyte-associated antigen (HLA) types an even stronger relationship between diabetes and the early introduction of cow's milk-based formula was observed (Kostraba *et al*. 1993; Perez-Bravo *et al*. 1996).

However, a recall bias of mothers is expected to strongly influence the outcome of such studies. Thus, prospective studies are required to elucidate the possible relationship between feeding patterns and the later development of autoimmunity, and the eventual progression to autoimmune disease. To date, two prospective studies have been

Abbreviations: HLA, human leucocyte-associated antigen; Th, T-helper.

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analysed regarding these questions. Neither study observed an association between the appearance of diabetes-related autoantibodies and the duration of exclusive or total breast-feeding, or with the time point at which cow's milk-based infant formulas, dairy products, or cow's milk itself were introduced (Couper *et al*. 1998; Hummel *et al*. 1998; Harrison & Honeyman, 1999). As yet, the prospective studies comprise only small numbers of cases, and therefore do not allow firm conclusions. Furthermore, the observation period of these studies is still too short, considering that the influence of dietary factors on disease development might well extend into puberty (Kolb & Pozzilli, 1999). Finally a dose–response relationship must be considered. In one study, at least, a more frequent emergence of diabetes-related autoantibodies was associated with high consumption of cow's milk in childhood, although this relationship was not valid for the progression to clinical type 1 diabetes mellitus (Virtanen *et al*. 1998).

In recent years the availability of two rodent models which spontaneously develop insulin-dependent diabetes mellitus has allowed the direct testing of cow's milk and certain constituent proteins for their diabetes-inducing potential. In several studies the avoidance of intact cow's milk proteins led to a reduced frequency of diabetes, while the addition of cow's milk to the rodent's diet resulted in an increased incidence of the disease (Elliott & Martin, 1984; Scott *et al*. 1985; Elliott *et al*. 1988; Issa-Chergui *et al*. 1988; Scott, 1996). The outcome of such dietary studies is obscured by the fact that cereal-based diets appear to induce an even higher frequency of diabetes (Malkani *et al*. 1997; Paxson *et al*. 1997). Second, cow's milk proteins are less diabetogenic in diabetes-prone BB rats than in non-obese diabetes mice (Scott, 1996).

A major advantage of animal models is that diets can be switched (diabetogenic *v*. non-diabetogenic) at different time points and dose–response relationships studied. From such experiments in the diabetes-prone BB rat it has become clear that switching diet type around puberty can still modify disease outcome, which suggests that the disease process is attributable to frequent exposure to a diabetes-promoting diet rather than to a single triggering event (Scott, 1996). However, as in human studies, the outcomes of such experiments are often highly variable, even in these inbred animal models (Scott, 1996). This variability raises the suspicion that a possible link between exposure to cow's milk and the later development of autoimmune diabetes might be under the control of other factors for which earlier studies have not been controlled (Kolb & Pozzilli, 1999). A summary of arguments in favour or against a role for dietary cow's milk protein in the pathogenesis of type 1 diabetes mellitus is listed in Table 1.

Table 1. Summary of findings supporting or arguing against a causal role of dietary cow's milk proteins in the development of type 1 diabetes mellitus

Pros	Cons
Population-based epidemiological studies	→ Not confirmed in all studies
Case–control studies	→ Several studies and trials have not found an association
Feeding experiments in animal models	→ Variable outcomes, other proteins are more 'diabetogenic'
Elevated antibody titres to cow's milk proteins in patients with type 1 diabetes mellitus	→ Major overlap between patients and controls
Elevated cellular immune response to cow's milk proteins in patients	→ Still methodological problems, HLA-matched controls required
Sequence homologies between cow's milk proteins and autoantigens in type 1 diabetes mellitus	→ Relevance not proven
Elevated antibody titres to bovine insulin in non-breast-fed infants	→ Substantial overlap, association with later type 1 diabetes mellitus remains to be proven

HLA, human leucocyte-associated antigen.

Possible mechanisms

Cow's milk contains five major proteins (% total proteins): caseins 70–80, β-lactoglobulin 10, α-lactalbumin 5, γ-globulin 2, bovine serum albumin 1. Antibodies against virtually all these proteins have been described in patients with recent-onset type 1 diabetes mellitus (Savilahti *et al*. 1988, 1993; Di Mario *et al*. 1988; Dahlquist *et al*. 1992; Krokowski *et al*. 1995; Wasmuth *et al*. 1995; Elliott *et al*. 1996; Füchtenbusch *et al*. 1997; Saukkonen *et al*. 1998), but only one study showed a high disease specificity of antibodies to bovine serum albumin (Karjalainen *et al*. 1992). Unfortunately, this study was challenged a few months later by another group (Atkinson *et al*. 1993; Lühder *et al*. 1994). In fact, almost all studies show a considerable overlap in antibody titres to most cow's milk proteins between subjects with diabetes and relevant controls. Furthermore, most of these studies have not tested HLA-matched control subjects, which seems mandatory in the investigation of an HLA-associated disease (Petrovsky & Harrison, 1995). This factor is further stressed by reports that there are also elevated levels of antibodies to cow's milk proteins in patients with selective immunoglobulin A deficiency (Buckley & Dees, 1969) and coeliac disease (Ferguson, 1977), both associated with DQ2 (A1*0501, B1*0201) (Hammarstrom & Smith, 1983; Saukkonen *et al*. 1996). This DQ allele is also present on the extended HLA haplotype known to confer risk of immune-mediated diabetes (Harrison & Honeyman, 1999).

As type 1 diabetes mellitus is thought to be a T-cell-mediated disease, cellular reactivity against cow's milk proteins might be more relevant. In fact, there have been reports linking an elevated response of unfractionated peripheral blood mononuclear cells to some cow's milk proteins to diabetes (Cheung *et al*. 1994; Cavallo *et al*. 1996; Vaarala *et al*. 1996). In some of these studies a major contribution of certain HLA haplotypes could be ruled out (Cavallo *et al*. 1996). However, none of these studies used patients with coeliac disease or immunoglobulin A deficiency as controls. Interestingly, in one study the cellular response to β-casein was elevated in patients with type 1 diabetes mellitus in comparison with (non-HLA-matched) controls, but similar when compared with their

siblings with a low risk of progression to overt diabetes (Ellis *et al*. 1998), which argues in favour of a non-strictly type 1 diabetes mellitus-associated genetic control of the immune response to dietary antigens.

One theoretical mechanism for the link between cow's milk exposure and autoimmune diabetes could be immunological cross reactivity (molecular mimicry) between cow's milk proteins and autoantigens of the β-cell. Indeed, sequence homologies between cow's milk proteins and islet autoantigens have already been identified by the use of computational sequence alignment (Kolb & Pozzilli, 1999). However, the relevance of these homologies has still to be shown at the level of autoreactive T-cells. Homologies to β-cell autoantigens have also been observed for other environmental factors, such as enteroviruses, (Graves *et al*. 1997; Honeyman *et al*. 1998).

An alternative concept of cross reactivity between cow's milk proteins and β-cells has recently been developed by Vaarala *et al*. (1998, 1999), who showed that infants with an early exposure to cow's milk-based infant formula have elevated antibody titres to bovine insulin, a normal constituent of cow's milk. Accordingly, early exposure to cow's milk would lead to immunization rather than to (normally occurring) tolerance to insulin, the only known β-cell-specific autoantigen in type 1 diabetes mellitus. This very interesting hypothesis awaits confirmation by prospective analysis of infants with antibodies to bovine insulin for later diabetes development.

A breakdown of tolerance mechanisms to dietary antigens might be promoted by cow's milk components with immunomodulatory properties. A major source of immunomodulatory compounds are the caseins. On digestion caseins give rise to a variety of peptides, some of which have strong opioid-like activities through μ-receptors on immunocompetent cells (Teschemacher *et al*. 1997). One of these peptides is β-casomorphin-7, which has been shown to have strong inhibitory effects on intestinal intra-epithelial lymphocyte proliferation (Elitsur & Luk, 1991). This peptide is exclusively released from β-caseins with a histidine residue at position 67 of the molecule (β-casein A1 and B; Jinsmaa & Yoshikawa, 1999). Interestingly, the *per capita* consumption of these genetic variants of β-casein significantly correlates with the national incidence of type 1 diabetes mellitus in children under 15 years of age (Elliott *et al*. 1999). It is possible that these peptides weaken tolerance mechanisms to other dietary antigens, including cow's milk proteins. Target cells of β-casomorphin-like peptides would be antigen-presenting cells of the gut or regulatory T-cells mediating oral tolerance (Weiner *et al*. 1994).

An alternative view of the role of cow's milk in autoimmune diabetes envisages that it is not the introduction of cow's milk but the lack of breast milk that might have detrimental effects in genetically predisposed individuals. Breast milk is a source of many cytokines and growth factors which have a role in the maturation of gut-associated lymphoid tissues (Srivastava *et al*. 1996). In contrast, cow's milk-based infant formulas are almost devoid of such factors in biologically active form (Kolb & Pozzilli, 1999). Equally important for the establishment of oral tolerance mechanisms seems to be the colonization of the gut with gram negative bacteria. Interestingly, optimal hygiene at birth and within the first few weeks has been shown to delay the normal colonization of the gut, giving rise to a predominantly aerobic flora which promotes gut inflammation and interferes with oral tolerance mechanisms (Kolb & Pozzilli, 1999). Indeed, it has been proposed that improved hygiene may contribute to the increasing incidence of autoimmune diabetes in the Western world (Kolb & Elliott, 1994).

There are several immune-mediated diseases for which disturbed oral tolerance mechanisms have been proposed, one of which is coeliac disease (Mäki & Collin, 1997; Feighery, 1999). Coeliac disease has been long known to be associated with type 1 diabetes mellitus, and up to 9 % of patients with type 1 diabetes mellitus have autoantibodies indicative of coeliac disease (Cronin & Shanahan, 1997). This association is suggested to be due to a similar genetic predisposition for both diseases (Catino *et al*. 1998). In coeliac disease wheat gluten is known to induce a pro-inflammatory mucosal response that later leads to autoimmunity. Interestingly, it takes more than 2 years before systemic autoimmunity is induced (Kolb & Pozzilli, 1999), a fact that has also to be considered when studying the role of dietary factors in the pathogenesis of type 1 diabetes mellitus.

A link between mucosal immunity and autoimmune diabetes is also suggested by the finding that islet-infiltrating T-cells express the integrin heterodimer α4β7, which is characteristically present on mucosal T-cells (Hänninen *et al*. 1993*b*, 1998). In a subsequent report it was shown that the depletion of cells bearing the α4β7 integrin leads to a reduced reactivity of T-cells to the autoantigen glutamate decarboxylase (Paronen *et al*. 1997). These findings support earlier experiments showing that a T-cell line isolated from the pancreas of a child with type 1 diabetes mellitus preferentially binds to the endothelium of both the pancreas and the appendiceal mucosa (Hänninen *et al*. 1993*a*).

All these findings imply that gut-associated tolerance mechanisms play a key role in the pathogenesis of immune-mediated diabetes (Fig. 1). In animal models of the disease there is increasing evidence that these regulatory mechanisms might be intrinsically disturbed and are particularly prone to further modulation through environmental factors (Goebel *et al*. 1999). Whether these assumptions hold true for type 1 diabetes mellitus in human subjects has yet to be shown.

Despite these uncertainties an international multi-centre trial has been set up to test the hypothesis that the avoidance of intact cow's milk proteins after prolonged breast-feeding in the first 9 months in genetically-predisposed infants might prevent the appearance of diabetes-associated autoantibodies or even type 1 diabetes mellitus itself (Akerblom *et al*. 1993). An interim analysis of this primary prevention trial suggests that delayed introduction of cow's milk products in the infant diet decreases the incidence of islet autoimmunity (Akerblom *et al*. 1999). However, full analysis of the study has to be awaited before drawing conclusions.

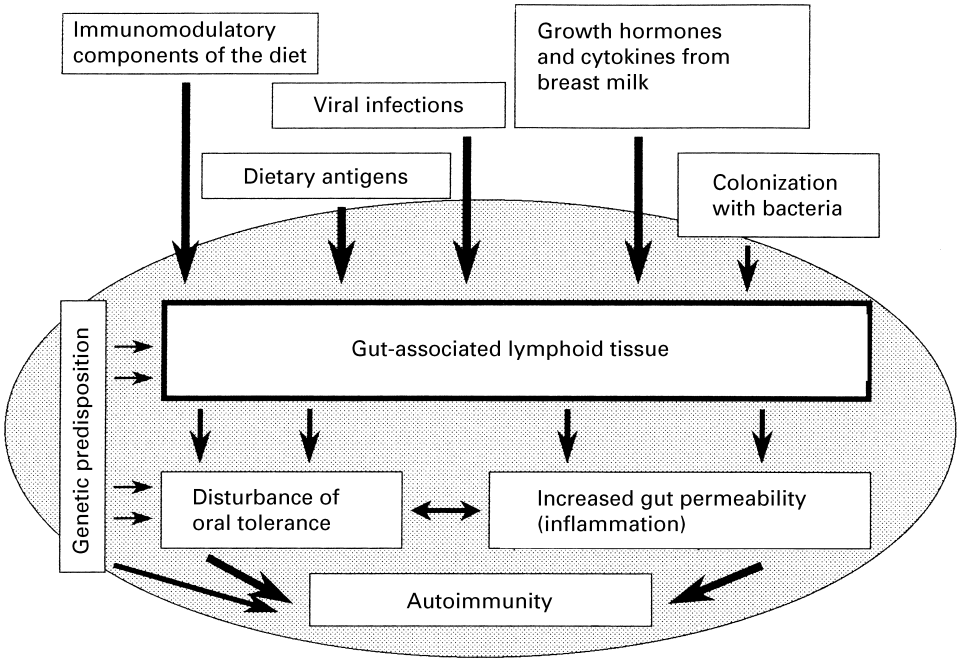

Fig. 1. Factors controlling gut maturation and normal function of the gut immune system. It is suggested that in type 1 diabetes mellitus a deficient oral tolerance to dietary components, including those of cow's milk, promotes islet inflammation and disease development.

Critical considerations

When examining the evidence for a possible role for cow's milk in the pathogenesis of type 1 diabetes mellitus it becomes clear that the link between them cannot be explained by a simple direct mechanism.

First, cow's milk-based diets have also been implicated in the aetiology of other immune-mediated diseases in human subjects, such as multiple sclerosis (Malosse *et al.* 1992), and have been shown to induce mild rheumatoid arthritis in genetically predisposed animals (Hanglow *et al.* 1985). There is a difference in the associations of these diseases with HLA, so that the association probably cannot be explained by the same mimicry epitopes in the different diseases. Furthermore, the consumption of cow's milk has also been associated with some other neurological diseases, e.g. autism (Lucarelli *et al.* 1995), some of which have not been proved to be immune mediated. Second, proteins other than cow's milk protein have been proved to be more diabetogenic in the available rodent models of diabetes. This factor is particularly true for some plant proteins, i.e. gluten and soyabean proteins (Scott, 1996) in the BB rat. Thus, attempts to just eliminate cow's milk from infant formulas and replace it by some other proteins might increase rather than decrease disease risk.

One factor that is special about cow's milk is that it is usually the first foreign dietary protein that encounters the maturating gut in newborn infants and young infants in the Western world. Depending on the genetic background of the individual and the stage of development of the gut-associated immune system, exposure to dietary protein may not elicit a balanced oral tolerance response. Hence, cow's milk proteins may have a stronger impact on gut immune functions than other food antigens. Oral tolerance reactions are usually dependent on a T-helper (Th) 2–Th3 cytokine secretion profile (Strobel & Mowat, 1998). Conversely, a pro-inflammatory Th1-biased cytokine profile is related to disturbed oral tolerance mechanisms (Garside *et al.* 1999). In recent years it has become clearer that in patients with type 1 diabetes mellitus there is a systemic bias towards Th1-type immune responses (Kallmann *et al.* 1997). This bias has not yet been confirmed for the gut-associated immune system, but reports about increased intestinal permeability in patients with type 1 diabetes mellitus (Carratu *et al.* 1999; Savilahti *et al.* 1999) also render such a Th1-biased pro-inflammatory immune response in this compartment to be highly probable.

In this scenario exposure to certain diets would promote disease development by two different pathways. First, some diets contain or give rise to biologically active constituents, e.g. casomorphins from caseins or other opioid-like peptides from wheat (Fukudome & Yoshikawa, 1992; Fukudome *et al.* 1997), which could further destabilize oral tolerance mechanisms in genetically predisposed individuals. Second, the diet provides antigens which might elicit an immune response to cross-reactive self antigens, thereby overcoming peripheral tolerance mechanisms (Kolb & Pozzilli, 1999). Such mimicry epitopes need not necessarily originate from dietary constituents, but could also be of viral or bacterial origin (Akerblom & Knip, 1998).

Taken together, the 'pathogenicity' of cow's milk, if it can be confirmed in man, appears to reside in an inappropriate gut immune response (Table 2). The quality of gut immune reactivity is influenced by the genetic predisposition, the duration of breast-feeding, the endoluminal micro-organisms and some biologically-active immunomodulatory components in the diet.

Table 2. Arguments in favour of shifting the focus from dietary antigens to the gut immune response

Critical considerations	Implications
Cow's milk may be associated with diseases other than type 1 diabetes mellitus	→ There is nothing special about cow's milk and type 1 diabetes mellitus
In animal models of type 1 diabetes mellitus cereal-based diets were more diabetogenic than cow's milk-based diets	→ Just replacing cow's milk by other proteins in the diet might cause more harm than benefit
The status of the gut-associated immune system is probably critical to a possible disease-inducing potential of dietary proteins, including cow's milk	→ More studies are needed to better define the status of gut immune reactivity after exposure to environmental challenges

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