

## Cow's Milk and Type 1 Diabetes

### The Real Debate Is About Mucosal Immune Function

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The hypothesis that early exposure of the infant to cow's milk (or lack of breast-feeding) predisposes the child to type 1 diabetes dates from the 1980s. It has important implications, but remains controversial because the evidence on which it is based has been indirect and is open to criticism. Two meta-analyses of multiple studies in which diabetes prevalence was associated retrospectively with infant feeding revealed only a marginal increase in relative risk. Two recent prospective studies found no apparent association between development of antibodies to islet antigens and feeding patterns in high-risk infants with a first-degree type 1 diabetic relative. Studies reporting increased humoral and cellular immunity to cow's milk proteins in children with type 1 diabetes often lack appropriate controls and standardization and do not, in themselves, establish a causal connection to disease pathogenesis. A review of published data leads to the conclusion that increased immunity to cow's milk proteins is not disease-specific, but reflects genetic predisposition to increased immunity to dietary proteins in general, associated with the HLA haplotype A1-B8-DR3-DQ2 (A1\*0501, B1\*0201), which also predisposes to celiac disease and selective IgA deficiency. We suggest that the cow's milk hypothesis could be productively reframed around mucosal immune function in type 1 diabetes. Breast milk contains growth factors, cytokines, and other immunomodulatory agents that promote functional maturation of intestinal mucosal tissues. In the NOD mouse model, environmental cleanliness may influence diabetes incidence through mucosal mechanisms, and exposure of the mucosa to insulin (present in breast milk) induces regulatory T-cells and decreases diabetes incidence. The mucosa is a major immunoregulatory barrier, and cow's milk happens to be the first dietary protein it encounters. The basic question is whether impaired mucosal immune function predisposes to type 1 diabetes. *Diabetes* 48:1501-1507, 1999

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BSA, bovine serum albumin; ICA, islet cell antibody; IEL, intestinal epithelial lymphocyte;  $\beta$ -LG,  $\beta$ -lactoglobulin; PBMC, peripheral blood mononuclear cell.

The influence of diet on diabetes incidence in the Bio-breeding (BB) rat and nonobese diabetic (NOD) mouse models and epidemiological studies in humans implicate exposure to cow's milk in infancy and/or a lack of breast-feeding as risk factors for type 1 diabetes. The American Academy of Pediatrics in 1994 and again in 1997 recommended breast-feeding and delayed introduction of cow's milk in infants with a family history of type 1 diabetes (1). However, without doubting the many benefits of breast milk, the evidence on which this recommendation is based can be questioned. The cow's milk debate nevertheless provides a platform on which to consider the possible linkage between genotype, mucosal immune function, and type 1 diabetes.

#### DIET AND RODENT MODELS OF TYPE 1 DIABETES

In the BB rat and NOD mouse, synthetic amino acid and casein hydrolysate diets were shown to be associated with a significantly lower incidence of diabetes than standard intact casein-containing diets (2-5). However, in the BB rat, addition of 25% casein as the only protein source (6,7), or bovine serum albumin (BSA) or whole cow's milk protein (8), did not reverse this protection. The studies of Scott (9) and associates now indicate that components from plants, and not cow's milk, specifically those from wheat and soya bean, are the major dietary diabetogens in the BB rat and exert an effect even when animals are first exposed after weaning. The role of plant products has not been clarified in the NOD mouse. Rather, Elliott et al. (10) have gone on to propose that an immunomodulatory peptide,  $\beta$ -casomorphin 7, derived from A1 casein, is diabetogenic. Addition of 10% A1 casein, but not A2 casein (which does not yield  $\beta$ -casomorphin 7), to a Prosobee soya bean-based diet increased diabetes incidence from 0 to 47%.  $\beta$ -Casomorphin 7 has been shown to be a  $\mu$ -opioid receptor agonist that may alter T-cell function (11). In support of this mechanism, Elliott et al. (10) observed that addition of the opiate receptor antagonist naloxone to the drinking water of NOD mice almost completely reversed the diabetogenic effect of A1 casein. This remarkable result awaits confirmation, and must be reconciled with evidence that other immunomodulatory peptides derived from milk, including human milk, have  $\mu$ -receptor agonistic properties.

#### INFANT FEEDING AND TYPE 1 DIABETES IN HUMANS

Several investigators have noted a high correlation between per capita consumption of cow's milk and the prevalence of

type 1 diabetes between (12,13) and within (14) countries. This observation relates to milk consumption across all ages, not just in infancy, and correlations at least as high are reported for coffee and sugar consumption (15). Against this backdrop, the seminal report of Borch-Johnsen et al. (16) in 1984 of an inverse relationship between breast-feeding frequency/duration and type 1 diabetes prevalence heralded a rash of over 20 similar studies, all but three strictly retrospective. In a meta-analysis of the first 13 studies, Gerstein (17) concluded that there was only a small protective effect of breast-feeding, the lack of which, or exposure to cow's milk, resulted in a relative risk no greater than 1.5. This was subsequently confirmed in a larger meta-analysis by Norris and Scott (18), who concluded that the apparent weak association could be explained by recall bias in retrospective studies or by disparate control groups. From an immunogenetic perspective, two of the studies analyzed may be instructive. Kostraba et al. (19) and Pérez-Bravo et al. (20) reported that the relative risks were higher, 11.3 and 13.1, respectively, in children with HLA susceptibility genes for type 1 diabetes who had early exposure to cow's milk or shorter periods of breast-feeding. Logically therefore, the question of cow's milk and type 1 diabetes should be resolved by prospective studies focused on individuals at highest genetic risk and designed to discriminate exposure to cow's milk from lack of breast-feeding.

Norris et al. (21), in the Denver-based Diabetes Autoimmunity Study in the Young (DAISY), retrospectively analyzed infant feeding patterns up to 6 months of age in relation to the development of islet autoantibodies up to 7 years of age. They found no significant associations. Recently, in a preliminary report from the Australian BabyDiab Study, Couper et al. (22) prospectively analyzed infant feeding patterns and the development of islet autoimmunity in high-risk infants. Newborns with a first-degree relative with type 1 diabetes were followed for a median of 29 (9–73) months. Home diaries recorded infant feeding, but no systematic feeding advice was given. Islet cell antibodies (ICAs), insulin autoantibodies, glutamic acid decarboxylase (GAD) antibodies and tyrosine phosphatase IA2 antibodies were measured every 6 months. Cox proportional hazards survival analysis revealed no association between infant feeding and detection of a single antibody once, a single antibody repeatedly, or two or more antibodies. This study analyzed the duration of exclusive and total breast-feeding, as well as the times at which infant for-

mula, dairy products, or cow's milk itself were introduced. The lack of a demonstrable association does not necessarily exclude the possibility that one may exist. However, the same result was also found in a preliminary report from the German BABY-DIAB Study Group (23). Full reports of these two prospective studies of at-risk infants and the results of an ongoing trial of nutritional intervention in at-risk infants in Finland are awaited, but at this time one would have to conclude that there is no evidence for an association between islet autoimmunity and cow's milk in high-risk infants.

#### IMMUNITY TO COW'S MILK PROTEINS IN CHILDREN WITH RECENT-ONSET DIABETES

The sources of cow's milk protein in infancy include dairy products that end up in maternal breast milk, hydrolyzed cow's milk protein infant formulae and supplements, dairy products such as custard, cheese, and yogurt, which are increasingly consumed worldwide, and cow's milk itself. Cow's milk contains five principal proteins: caseins (70–80%),  $\beta$ -lactoglobulin ( $\beta$ -LG) (10%),  $\alpha$ -lactalbumin (5%),  $\gamma$ -globulin (2%), and BSA (1%). Its protein content is higher than that of human milk because of the higher concentration of casein, and  $\beta$ -LG is not present in human milk. IgG antibodies to cow's milk proteins are present in virtually all infants exposed to cow's milk (24,25) and have been considered physiological (25). The titers of antibodies to cow's milk proteins decrease with age (24), but are higher in celiac disease (24) and selective IgA deficiency (26). These two disorders are associated with HLA-DQ2 (A1\*0501, B1\*0201) (27–29), which is present on the extended HLA haplotype A1-B8-DR3, as well as A30-B18-DR3, well known to also confer risk for type 1 diabetes (30).

One mechanism proposed for an association between cow's milk exposure and type 1 diabetes is immunological cross-reactivity (molecular mimicry) between cow's milk proteins and islet autoantigens. Therefore, before reviewing the evidence for immunity to cow's milk proteins in type 1 diabetes, we might consider criteria by which an autoantigen or cross-reactive nonself antigen could be evaluated for a pathogenic role (Table 1). These criteria, centered on the autoantigen, extend those previously proposed for defining autoimmune diseases (31). In the case of type 1 diabetes, proinsulin/insulin, GAD, and tyrosine phosphatase IA2 are the only autoantigens that partially satisfy these criteria in rodents or humans. If cow's milk proteins are proposed to trig-

TABLE 1  
Criteria for an autoimmune disease: pathogenicity of the autoantigen or cross-reactive antigen

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Immune responses to the antigen are disease-specific.
Immune responses to the antigen precede the onset of disease.
Immune responses to the antigen reflect disease pathology, i.e., are surrogate markers.
Antigen-specific antibodies or T-cells mediate/transfer disease.
Immunization with antigen reproduces disease.
Antigen peptides co-purify with disease-associated major histocompatibility complex molecules.
Manipulation of antigen expression modifies disease expression.
Administration of antigen via tolerizing mode or route prevents disease.

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ger or exacerbate islet autoimmunity, these criteria should be applied to them.

**Humoral immunity.** Antibodies to BSA in the sera of children with type 1 diabetes were first noted in 1987 (32). They reacted with BSA in fetal calf serum adventitiously labeled by <sup>35</sup>S-methionine on the surface of islet cells during attempts to immunoprecipitate autoantigens. However, on follow-up, there was no significant difference overall in the levels of BSA antibodies between children with recent-onset type 1 diabetes and control groups matched for age and socioeconomic status. Karjalainen et al. (33) reported that BSA antibodies were increased in 100% of recent-onset type 1 diabetic patients, but this was not confirmed by Atkinson et al. (34), Lühder et al. (35), Ivarsson et al. (36), or Fuchtenbusch et al. (37), who, like ourselves originally (32), found considerable overlap with control subjects, and especially with siblings who would share HLA alleles with their diabetic probands. These studies clearly do not demonstrate specificity of BSA antibodies for type 1 diabetes.

Savilahti and associates (38,39) for the Childhood Diabetes in Finland Study Group reported increased levels of IgA to whole cow's milk proteins and bovine  $\beta$ -LG and increased levels of IgG to  $\beta$ -LG in recent-onset patients, especially of younger age, compared with those in age-matched control subjects. They suggested that the higher levels of antibodies in younger children were not explained only by a lack of breast-feeding. However, they also found that nondiabetic siblings had increased levels of IgA antibodies to cow's milk proteins. Dahlquist et al. (40) reported that IgA antibodies to bovine  $\beta$ -LG were an independent determinant of diabetes risk in Swedish children. Recently, Saukkonen et al. (41), for the Finland Study Group, reported increased IgG antibodies to BSA (not previously reported in their studies), as well as to  $\beta$ -LG and whole cow's milk proteins, and increased IgG antibodies to whole cow's milk proteins in childhood diabetes. Importantly, the control group comprised nondiabetic siblings matched for HLA DQ B1 alleles. DQB1\*0201 itself was associated with increased IgG antibodies to BSA, independent of infant feeding patterns or recent cow's milk intake. Krokowski et al. (42) had earlier found an association between increased levels of BSA antibodies and HLA DR3. Specific HLA alleles and haplotypes are known to be preferentially associated with stronger humoral or cellular immunity to various antigens, including islet and other autoantigens (43–45). The HLA A1-B8-DR3-DQ2 haplotype, for example, is associated with stronger humoral immune responses to GAD (43). Thus, claims of disease-specific immunity to cow's milk proteins cannot be substantiated without controlling for HLA status.

**Cellular immunity.** If immunity to cow's milk proteins has relevance, cellular immunity should be more disease-specific, given that type 1 diabetes is T-cell-dependent. Proliferative responses of peripheral blood mononuclear cells (PBMCs) to BSA,  $\beta$ -LG, and  $\beta$ -casein have been reported. Cheung et al. (46) found increased responses to BSA and to a 17-amino acid albumin bovine serum (ABBOS) peptide (amino acids 152–168) of BSA in recently-diagnosed children, but in contradistinction to Atkinson et al. (34). Vaarala et al. (47) found increased responses only to  $\beta$ -LG, not BSA,  $\alpha$ -casein, or ovalbumin, in recently-diagnosed children as a group, but observed considerable overlap with unrelated control subjects. Cavallo et al. (48) found increased responses to  $\beta$ -casein in recent-onset patients compared

with non-HLA-matched control subjects. On the other hand, while Ellis et al. (49) also found increased responses to  $\beta$ -casein in recent-onset patients compared with islet autoantibody-negative healthy control subjects, responses in the patients and their autoantibody-negative first-degree relatives were almost identical. This not only indicated the necessity for HLA matching, but supported their previous contention (34) that genetic predisposition to autoimmune disease may be associated with a defect in tolerance to dietary antigens, which has been echoed subsequently by Vaarala et al. (47) and Åkerblom and Knip (5). The discrepancies among these cellular immune studies strongly suggest, as cautioned previously (50), that T-cell responses dependent on and restricted by HLA molecules can be evaluated only by comparison with HLA-matched control subjects. Admittedly, this is not always easy. Our solution has been to seek consent for blood donation from individuals who are registered as potential bone marrow donors.

Apart from lack of HLA-matched control subjects, studies of human cellular immune function, including those referred to above, suffer from a lack of standardization in other ways that prevents evaluation and comparison. First, quality control data on the purity of putative cow's milk protein antigens are lacking. Second, testing of other antigens, both nonself and self, has not been routine, but is essential to determine whether an increased response to a cow's milk protein is specific or reflects a more general hyperimmune state. Third, exogenous factors, e.g., the time of day at which blood is taken (51), may influence cellular immune function in peripheral blood and should be standardized. Fourth, most reports provide no data on within- and between-assay reproducibility. Finally, for proliferative responses of PBMCs, raw data should be provided so that the significance of derived stimulation indices can be evaluated.

We examined proliferative responses of PBMCs to several cow's milk components in at-risk islet autoantibody-positive first-degree relatives compared with HLA-matched control subjects (Table 2). Stimulation indices were not significantly different between the groups. Stimulated responses were increased in some at-risk relatives, but concomitantly with an increase in basal activity, resulting in no increase in the derived stimulation index compared with control subjects. The increased absolute basal and antigen-stimulated responses in these at-risk relatives may reflect a state of general hyperimmunity previously observed to precede the onset of clinical diabetes in a prospective study of at-risk relatives (52). However, this observation is not easy to confirm from published cross-sectional studies of at-risk relatives or recently-diagnosed patients because HLA-matched control subjects, basal activity data, or both are missing. Thus, in reviewing 46 T-cell proliferation studies in at-risk relatives or recently-diagnosed patients reported since 1975 (data available on request; L.C.H., M.C.H., unpublished observations), only 12 reported raw data that included control subjects; only 15 included HLA-matched or HLA-stratified control subjects, and in these, basal activity data were reported in only three. Given the dependence of basal (autologous) T-cell proliferation on HLA class II (53) and of stimulated activity on the well-known HLA class II restriction of antigenic peptides, it would seem spurious to make comparisons with non-HLA-matched control subjects.

The unconfirmed report of increased PBMC proliferative responses to BSA/ABBOS peptide has been extended by the

TABLE 2  
Proliferative responses of PBMCs to cow's milk proteins

	<i>n</i>	Basal	Tetanus toxoid	β-Casein	κ-Casein	Fat globule protein	BSA
Islet antibody-positive first-degree relatives	12						
[ <sup>3</sup> H]thymidine uptake (counts/min)		1,772 ± 2,020	17,879 ± 11,592	4,436 ± 3,742	3,090 ± 2,439	6,468 ± 3,845	1,382 ± 988
Stimulation index		—	24 ± 35	3.7 ± 1.6	3.2 ± 2.2	7.7 ± 8.9	1.1 ± 0.4
HLA DR-DQ-matched control subjects	12						
[ <sup>3</sup> H]thymidine uptake (counts/min)		476 ± 416	5,836 ± 4,616	1,396 ± 1,161	1,407 ± 1,938	4,356 ± 3,850	710 ± 911
Stimulation index		—	26 ± 24	3.7 ± 3.5	4.7 ± 7.2	9.4 ± 5.5	1.3 ± 0.6

Data are means ± SD. PBMCs were separated from heparinized venous blood by centrifugation through Ficoll Paque and resuspended at 10<sup>6</sup> cells/ml in RPMI 1640 medium containing 20 mmol/l HEPES, 10% autologous serum, and 10<sup>-5</sup> mol/l 2-mercaptoethanol. A 200-μl cell suspension was added in replicates of six to U-bottom wells of a 96-well tissue culture plate, with or without antigen at a final concentration of 16.7 μg/ml or 20 Lyons flocculating units/ml for tetanus toxoid. After incubation at 37°C in 5% CO<sub>2</sub> for 6 days, cells were pulsed with 0.25 μCi/well [<sup>3</sup>H]thymidine and harvested after 6–8 h for scintillation counting. The median counts per minute of replicate wells was determined and used for the calculation of group means. Antigens were sourced as follows: preservative-free tetanus toxoid, Commonwealth Serum Laboratories, Melbourne, Australia; β- and κ-casein, Department of Primary Industries Laboratory, Brisbane, Australia; fat globule protein, in-house preparation; and BSA, Sigma, St. Louis, MO. Protein antigen purity was checked by SDS-PAGE analysis. Endotoxin in antigen preparations was measured in the Limulus lysate assay (Kinetic-QCL 192 Test Kit; Bio-Whittaker, Walkersville, MD) and ranged in final concentration in PBMC assays from 280 to 740 pg/ml.

original investigators to encompass cross-reactivity with a putative ICA69 (54). The ICA69 sequence is said to induce T-cell anergy, which can be overcome by cross-reactivity with the ABBOS peptide in BSA. Other considerations aside, the relevance of this to type 1 diabetes is unclear. Importantly, Lampasona et al. (55) were not able to confirm that ICA69 is a type 1 diabetes-specific autoantigen, and Ronningen et al. (56) have recently shown that there is no cross-reactivity between BSA and ICA69 at the antibody level. Claims and counter-claims in this area will continue as long as investigators neglect criteria by which to evaluate the potential pathogenicity of an antigen, fail to use standardized protocols and appropriate controls for subjects and antigens, and, in regard to molecular mimicry, fail to provide biological evidence, i.e., epitope cross-reactivity at the T-cell level.

A direct link between islet antigen-reactive T-cells and mucosal immune function in NOD mice was made by Hänninen et al. (57), who showed that the integrin heterodimer α4β7, which is characteristically expressed by mucosa-derived T-cells, is present on most infiltrating T-cells in the insulinitis lesion and that its receptor, the mucosal addressin MAdCAM-1, is induced on endothelium within and adjacent to the islet lesion. Furthermore, treatment of young mice with antibody to MAdCAM-1 prevented the development of diabetes (58). In humans, they found (59) that a T-cell line derived from the pancreas of a child with recent-onset diabetes preferentially adhered to endothelium of both pancreas and appendiceal mucosa. Subsequently, Paronen et al. (60) reported that reactivity of circulating T-cells to GAD in type 1 diabetic patients markedly decreased after depletion of α4β7 integrin-positive cells. As noted by these investigators, the finding that islet antigen-reactive T-cells express a mucosa homing molecule whose vascular addressin is present in islets implicates mucosal immunity in the pathogenesis of type 1 diabetes.

#### BREAST MILK LACK, COW'S MILK, AND TYPE 1 DIABETES: POSSIBLE MECHANISMS

Some of the possible consequences of breast milk lack and/or early introduction of cow's milk (or other dietary components) are suggested in Table 3. It is reasonable to assume, in the infant at least, that the consequences of a lack of exposure to breast milk and/or the exposure to foreign dietary proteins will reflect the competence of the intestinal mucosa and mucosa-associated immune system. Breast milk contains a host of growth factors and cytokines, mostly species-specific, many of which appear to have a role in the maturation of intestinal mucosal tissues (61,62). Could their lack impair the development of mucosa-mediated tolerance to islet autoantigens, such as insulin in breast milk? Oral (mucosa-mediated) tolerance refers to the suppression of systemic cellular (T-cell) immunity and IgE humoral immunity to an antigen after delivery to the mucosa of the antigen in soluble form (63,64). Immunoreactive insulin is readily detectable in human breast milk at stable concentrations of up to 5 ng/ml. (Pro)insulin is the only β-cell specific autoantigen in type 1 diabetes, and an increasing body of evidence indicates that it plays a key role in driving β-cell destruction. In the NOD mouse, transgenic expression of proinsulin in antigen-presenting cells completely prevents diabetes (65), and oral (66), aerosol (67), or intranasal (68) insulin induces regulatory T-cells associated with suppression of diabetes incidence. NOD mice maintained under germ-free conditions have a high incidence of diabetes that is reduced by conventional conditions of housing and feeding (69). Under the latter, bacterial colonization of the intestine is accompanied by an increase in the numbers of intestinal epithelial lymphocytes (IELs), particularly CD8 γδ T-cells, and by maturation of mucosal immune function (70,71). The regulatory T-cells induced by aerosol (67) or

TABLE 3  
Cow's milk and insulin-dependent diabetes: potential mechanisms

Breast milk lack	Cow's milk (or other dietary components)
Lack of growth factors and cytokines for maturation of mucosa and mucosa-associated lymphoid tissue.	Immunization to dietary antigens cross-reactive with pancreatic islet autoantigens, e.g., bovine insulin with human insulin (76), bovine $\kappa$ -casein with tyrosine phosphatase IA-2, wheat and soya bean NADH ubiquinone reductase with tyrosine phosphatase IA-2 (78).
Impaired mucosa-mediated tolerance to pancreatic islet autoantigens, e.g., human insulin in breast milk, or to cross-reactive epitopes (? accentuated by genotype, e.g., HLA A1-B8-DR3-DQ2).	
Lack of passively-transferred maternal immunity (IgA, IgG, IgM, leukocytes, lysozyme, lactoferrin, cytokines).	Immunomodulatory cow's milk peptides, e.g., $\beta$ -casomorphins (11).
Predisposition to potentially diabetogenic enteric infections, e.g., enterovirus, rotavirus.	Impaired mucosal immunity.

intranasal (68) insulin are CD8  $\gamma\delta$  T-cells characteristic of IELs. Finally, it has been shown that antibody-mediated inhibition of  $\gamma\delta$  T-cell function in vivo abrogates oral tolerance (72,73) and the induction of regulatory CD8  $\gamma\delta$  T-cells by aerosol insulin (68). Together, these findings imply that the state of the mucosa, conditioned by the environment, is a critical determinant in susceptibility to diabetes in NOD mice and, by inference, to type 1 diabetes in humans.

Generation of regulatory cells to insulin (in breast milk) may be a normal developmental process. Impaired maturation of mucosa-associated lymphoid tissue could lead to failure of this process and to immunization rather than tolerization. In neonatal mice, the ontogeny of oral tolerance induction is strain-dependent with a clear temporal profile. Intra-gastric administration of antigen during the first 7–10 days of life generally does not suppress, and in fact may prime for, systemic immunity (74,75). Alternatively, Vaarala et al. (76) have proposed that bovine insulin in cow's milk may generate cross-reactive immunity to human insulin. Although bovine and human insulins differ by only three amino acids, bovine insulin is known to be immunogenic in humans (77). With regard to other islet antigens, we have found sequence identity/similarity between bovine  $\kappa$ -casein and a T-cell epitope in tyrosine phosphatase IA2, and between NADH ubiquinone reductase in wheat and soya bean and a different epitope in tyrosine phosphatase IA2 (78), but at this stage we have no evidence for cross-reactive immunity between these peptides.

Many studies have shown that breast milk protects against enteric infections (79). It would be important, therefore, to consider enteric infections, particularly with potentially diabetogenic viruses such as Coxsackie (80) and rotavirus (78), in evaluating infant feeding and diabetes risk. Furthermore, enteric infections could increase immunity to dietary antigens by increasing intestinal permeability or by perturbing mucosal immunity.

In addition to the possible consequences of breast milk lack (Table 3), individuals genetically at-risk for type 1 diabetes,

e.g., with the HLA A1-B8-DR3-DQ2 haplotype, may have impaired mucosa-mediated tolerance and, consequently, increased immunity to many dietary proteins both self (e.g., human insulin) and nonself (cow's milk or plant proteins). This concept is supported by the association of type 1 diabetes with clinical and subclinical celiac disease (28,81), through this HLA haplotype, and between celiac disease and increased immunity to dietary proteins (24,25).

#### CONCLUDING REMARKS

Available data do not allow the conclusion that cow's milk itself is pathogenic in type 1 diabetes. Increased immunity to cow's milk proteins may occur in selected individuals with susceptibility to type 1 diabetes, but is likely to reflect impaired function of mucosa-associated lymphoid tissue. The latter may result from or be accentuated by lack of growth factors and cytokines present in human milk. In addition, certain individuals, e.g., those with the HLA A1-B8-DR3-DQ2 haplotype, may be predisposed to sensitization to dietary antigens and to autoimmune disease because of impaired mucosa-mediated immunoregulation. It follows, therefore, that cow's milk is not unique, but simply the first dietary antigen encountered, and that predisposed individuals may also exhibit increased immunity to substitutes such as goat and soya milk. Potential mechanisms (Table 3) are worthy of further investigation not only because the cow's milk hypothesis has important medical and social implications, but because of the probable link between mucosa-mediated immunoregulation and type 1 diabetes.

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