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Review

The role of the gut in β-cell autoimmunity and type 1 diabetes: a hypothesis

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Abstract: The origin of autoimmunity leading to the destruction of insulin-producing β-cells is not known. Several studies suggest that a link exists between the gut immune system and the islets infiltrating lympocytes. Inflamed pancreatic islets express the same adhesion molecules involved with the homing of gut-associated lymphocytes. The manifestation of autoimmune diabetes in the animal models can be modified by dietary factors, which cause changes in the cytokine production by islet-infiltrating lymphocytes. Increased risk of type 1 diabetes has been associated with an early introduction of cows' milk formula in infancy, indicating that triggering of the gut immune system in early infancy may contribute to the later development of β-cell autoimmunity. Enhanced immune reactivity to cow milk (CM) proteins in the patients with type 1 diabetes suggests aberrant regulation of the gut immune system in this disease. In the patients with newly diagnosed type 1 diabetes, anti-glutamate decarboxylase (GAD)-reactivity was found in the subpopulation of lymphocytes expressing gut-associated homing receptor α4β7. Based on these findings, the hypothesis that aberrant function of the gut immune system would lead to the development of β-cell autoimmunity and type 1 diabetes has recently received a lot of attention. The possibility that regulation of the gut immune system is not normal in subjects at risk of autoimmune diabetes should be considered when treatments interfering with mucosal immunity for the prevention of type 1 diabetes are planned.

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Key words: cow milk – enterovirus – gut immune system – insulin – type 1 diabetes – oral tolerance

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Type 1 diabetes is considered a T-cell mediated autoimmune disease in which autoreactive T lymphocytes infiltrate the islets of pancreas and destroy the insulin-producing β-cell population. Autoimmunity to several β-cell antigens, such as insulin, glutamate decarboxylase (GAD), islet cell antigen 69kD (ICA69) and islet antigen 2 (IA-2), occurs in patients with type 1 diabetes and often precedes hyperglycemia. Environmental factors modify the development of this disease, although the role of genetic factors in the pathogenesis is strong. The triggers of autoimmunity against β-cells are poorly identified. The epidemiological association of the risk of type 1 diabetes and exposure to the environmental risk factors, which stimulate the gut immune system, namely enteroviral infections and cow milk (CM) proteins, suggests a link between the gut immune system and the pathogenesis of type 1 diabetes (1). The studies searching for dietary triggers of diabetes support the view that changes in the gut immune system influence the development of autoimmune diabetes (2, 3). The experimental studies in non-obese diabetic (NOD) mice, an animal model of autoimmune diabetes, have suggested that the tissue-specific homing properties of lymphocytes could provide the immunological link between the gut and inflamed pancreatic islets (4–6). Based on these observations, the interest of diabetes research has been focused recently on the possible aberrant function of gut immune system in the subjects at risk of type 1 diabetes.

The effect of diet on the development of autoimmune diabetes

The effect of diet on the development of autoimmune diabetes has been studied in the two animal

models of type 1 diabetes. The classical study by Elliot and Martin demonstrated that the incidence of diabetes in biobreeding (BB) rats was decreased when the diet containing amino acids, instead of whole proteins, was introduced after weaning (2). A similar kind of protective effect by hydrolyzed casein as the source of dietary protein after weaning has also been observed in NOD mice (3). Later, it was demonstrated that the use of hydrolyzed proteins as the source of (foreign) proteins in the diet resulted in a switch of the cytokine profile in the lymphocytes infiltrating the islets in BB rats (7). The diet of hydrolyzed casein did not prevent the insulitis, but the lymphocytes infiltrating the islets showed a functional profile with lowered cytotoxic activity demonstrated as increased expression of IL-4, IL-5 and IL-10 and decreased expression of IFN-γ. This is key evidence showing that changes in the diet may modify the functional properties of the lymphocytes infiltrating the pancreas. Karges et al. showed that the use of hydrolyzed casein in the weaning diet of NOD mice induced T-cells, which prevented diabetes in an adoptive transfer model (8). Also the exposure to dietary soy proteins and wheat gluten seems to modify the incidence of diabetes in both BB rats and NOD mice (9, 10). The development of autoimmune diabetes in these animal models is not dependent on the exposure to dietary CM proteins or soy proteins, because the animals develop diabetes spontaneously despite diets free of these protein (9, 11, 12). However, these studies clearly demonstrate that the development of autoimmune diabetes is modified by environmental factors, such as dietary factors, which cause detectable immunological changes in the pancreatic islets.

In humans, the exposure to CM formulas in early infancy has been associated with the risk of type 1 diabetes in several epidemiological studies (1). Epidemiological data suggest that CM exposure in early life (less than 3 months of age) may increase about twofold the risk of type 1 diabetes later in life. However, the epidemiological evidence is contradicting, as described in the review by Åkerblom and Knip (1). The interpretation of the epidemiological data is difficult because the consumption of CM is such a common phenomenon and the genetic factors, as well as other environmental factors, may modify the possible diabetogenic effect of dietary factors. The possible beneficial effect of breast milk feeding may confound the studies when the risk of early CM formula feeding is analyzed. The antigens and cytokines in breast milk may modify the development of immune response to food antigens (13). Accordingly, the importance of CM consumption as a risk factor of type 1 diabetes may vary in different studies owing to the genetic and environmental differences between the populations studied. An example of the complicated relation between an environmental trigger and a disease is found in celiac disease. Although dietary wheat gluten is evidently the trigger of celiac disease, and removal of dietary gluten leads to cure the villous atrophy, we do not know how exposure to wheat gluten (the age of introduction or the amount of protein in the diet) modifies the development of celiac disease. The results from the on-going large prospective birth-cohort studies are needed to answer the question of whether early infant feeding with CM-based formula is a risk of type 1 diabetes.

The association of early exposure to CM formula and the risk of type 1 diabetes has stimulated pilot studies on the dietary prevention of type 1 diabetes. Two pilot studies on the nutritional primary prevention of type 1 diabetes by elimination of CM proteins during the first 6–8 months of life (Trial to Reduce IDDM in the Genetically at Risk (TRIGR) study) have been performed in infants at genetic risk of diabetes (14, 15). The results of the second pilot of the TRIGR study are promising, because by the age of 2 yr the appearance of islet cell autoantibodies was lower in children who received casein hydrolysate formula than in children who received CM-based formula during the first 6–8 months of life (15). When more prospective studies are carried out in this field, the possible role of CM exposure in the pathogenesis of type 1 diabetes may become better understood.

In a prospective follow-up study of healthy siblings of diabetic children, the use of liquid CM was associated with the development of diabetes-associated autoantibodies and type 1 diabetes (16). This study suggested that the risk associated with the consumption of CM is not restricted to early infancy. In addition, the amount of CM consumed may have an effect on the diabetes risk because drinking three or more glasses of CM implied an increased risk of the development of diabetes-associated autoantibodies (16). The putative diabetogenic factors in CM are not known, but several candidates have been identified, as discussed later in 'Immune responses to oral antigens in type 1 diabetes'.

The dietary factors may influence the development of autoimmune diabetes also by unspecific mechanisms. The introduction of CM proteins to the infant's diet is a strong immunological stimulation (17, 18). It induced antigen-specific systemic humoral and cellular immune responses (17). An early start of CM formula led to the development of stronger antibody and T-cell responses to CM proteins than later introduction of CM proteins in the diet (17). Also, more general immunological changes, such as increased levels of plasma soluble adhesion molecules, were detectable in the infants exposed to CM formula (18). Early stimulation of the gut-associated immune system by foreign proteins may prime the gut immune system and lead to the development of functionally

different kinds of gut immune cells than later exposure.

Gut immune system in type 1 diabetes

The gut immune system is a major T-cell organ. It has been estimated that the number of small intestinal intraepithelial lymphocytes is more than half of the T-cell number estimated for peripheral lymphoid organs. The gut immune system can be divided into three major compartments: organized gut-associated lymphoid tissue (GALT) such as the Peyer's patches, the mucosal lamina propria and the epithelium. The activation of naive T-cells takes place in GALT, the differentiation of the activated lymphocytes in the mesenteric lymph nodes from where the lymphocytes circulate to the peripheral circulation. The circulating lymphocytes, which have been primed in the gut immune system, express a gut-specific homing receptor α4β7-integrin on their surface. This homing receptor directs the gut-associated memory cells back to the mucosal tissues. The tissue-selective homing is based on the interaction of the α4β7-integrin with the mucosal vascular addressin, MAdCAM-1, which is expressed in the mucosal lympoid tissue (19).

In type 1 diabetes, the origin of islet cell autoimmunity is not known. Extravasation of lymphocytes from the circulation into the target tissue is a multistep process, involving recognition and specific interaction of several adhesion molecules on the lymphocyte with their endothelial ligands. MAdCAM-1 is expressed in the inflamed islet of NOD mice, and the infiltrating T-cells express the same adhesion molecules as gut-associated lymphocytes, namely α4β7-integrin (4–6). The importance of MAdCAM-1/α4β7 interaction in the binding of the lymphoid cells to the pancreatic vascular endothelium has also been shown in functional studies (20). In NOD mice, the treatment from age 7 to 29 d or 8 to 12 weeks with monoclonal antibody against β7 integrin or MAdCAM-1 resulted in long-standing protection of diabetes and insulitis (21, 22). In a study by Hänninen et al. the blockade of the function of MAdCAM-1 by monoclonal antibodies reduced the incidence of diabetes when started at 3 weeks of age in NOD mice (22). The same treatment also inhibited diabetes and the homing of the lymphocytes into the pancreas in an adoptive transfer performed in NOD/severe combined immunodeficient (SCID) recipients (22). In the adoptive transfer model, lymphocytes derived from the mesenteric lymph nodes of young NOD donors were diabetogenic (22). These studies suggest that islet-cell reactive lymphocytes share the lymphocyte and endothelium adhesion molecules involved in the migration of lymphocytes into mucosal lymphoid tissues. The study by Hänninen et al. also indicates a role for MAdCAM-1 as a mucosal addressin involved in the initiation of diabetogenic autoimmunity in young NOD mice (22). Accordingly, β-cell autoreactive lymphocytes may belong to the compartment of gut-associated lymphocytes and may even originate from the gut mucosa. Supporting the latter hypothesis, the mesenterial lymphocytes derived from 3-week-old NOD mice have been shown to transfer diabetes (22, 23).

Also, some studies in humans have indicated that mucosal lymphocytes may be involved in the pathogenesis of type 1 diabetes (24, 25). When the endothelial cell-binding properties of a T-cell line derived from a diabetic pancreas were studied, a strong adherence to the endothelium of diabetic pancreas and mucosal lymphoid tissue but weak binding to endothelium of peripheral lymph node and normal pancreas was observed (24). We have demonstrated that GAD-reactive lymphocytes in patients with newly diagnosed type 1 diabetes express the gut-specific homing receptor α4β7-integrin, whereas tetanus toxoid (a parenteral antigen) reactive lymphocytes expressed low levels of α4β7 (25). In the same way, an antigen-specific T-cell response was found in the α4β7 positive T-cell population in rotavirus infection, indicating that α4β7+ memory T-cells show reactivity for intestinal antigens (26). The α4β7-expression on the islet cell antigen reactive lymphocytes suggests that autoreactive lymphocytes show homing properties to the intestinal lymphoid tissues and emphasizes the role of the gut immune system in the development of type 1 diabetes (25). It also indicates that tolerance to a self-antigen is broken in the gut-associated lymphocyte population in type 1 diabetes.

Interestingly, aberrant findings in gut immunohistology have been reported recently in type 1 diabetes by Savilahti et al. (27). They studied the immunohistology of the intestine in 26 patients with type 1 diabetes, 13 of whom had the human leukocyte antigen (HLA) DQB1*02 gene and increased risk of celiac disease. The results suggest that the structurally normal intestine of the patients with type 1 diabetes shows a stage of immune activation. Villous structure and the density of the intraepithelial lymphocytes were normal, but the extent of positivity with anti-DR (an HLA class II antigen) and -DP (an HLA class II antigen) antibodies in the villous epithelium was increased in the patients when compared with controls. Also, the densities of T-cell subtypes were similar in the patients and controls, but the patients had increased intensity of α4β7-expressing cells in the lamina propria. The findings were not restricted to the patients who carried the HLA DQB1*02 allele, suggesting that activation of the gut immune system may be associated with type 1 diabetes and does not associate only with the genetic risk allele shared with celiac disease. Besides markers of immune activation, increased intestinal permeability to mannitol has

been reported in patients with uncomplicated type 1 diabetes (28).

Studies on the functional characteristics of the gut-associated lymphocytes in type 1 diabetes are rare. In our preliminary studies, we have studied the secretion of IFN-γ, IL-4 and TGF-β by α4β7-expressing lymphocytes in patients with type 1 diabetes and healthy children. The ratio of IFN-γ secretion by α4β7 high vs. α4β7 low lymphocytes was increased in the patients with type 1 diabetes and the secretion of IFN-γ did not show inverse correlation with TGF-β secretion as in healthy children. These observations on the aberrant cytokine profile in lymphocytes divided into subpopulations by the expression of α4β7-integrin suggest a possible functional difference in the gut associated lymphocytes between patients with type 1 diabetes and healthy children (29, Klemetti et al., unpublished data).

Immune responses to oral antigens in type 1 diabetes

The development of autoimmunity is considered as a manifestation of broken immune tolerance to self-antigens. The induction of tolerance to self-antigens is controlled by the central tolerance, which means clonal deletion of T-lymphocytes encountering the self-antigens in the thymus. This process of negative selection is not complete and T-cells recognizing self-peptide/major histocompatibility complex (MHC) complexes migrate out from the thymus. The role of central tolerance in preventing the T-cell mediated autoimmunity is, however, restricted to self-antigens that are processed and presented by antigen-presenting cells in the thymus.

Peripheral tolerance controls tolerance to the peripheral antigens, which are not expressed in the thymus, or to cryptic self-antigens, which are modified or exposed only outside the thymus. Oral tolerance is a major compartment of peripheral tolerance and develops to antigens encountered in the gut (30). The gut immune system has a dual role: it provides defense against infectious agents, but also induces tolerance to harmless food and microbial antigens encountered in the gut. The molecular mechanisms of the induction of oral tolerance are poorly understood. In animal models, several studies indicate that oral antigen feeding induces tolerance in adult animals, but may prime the systemic immunity in neonatal animals. A wide range of antigen doses can induce oral tolerance; lower doses are needed for suppression of cell-mediated immunity when compared with humoral immunity. At doses below the tolerogenic window, feeding antigen may prime the immune response without causing systemic immunity. The different doses of oral antigens seem to induce oral tolerance by at least partly different mechanisms. High doses of oral antigen induce the apoptotic deletion of antigen-specific cells in the Peyer's patches, whereas low doses induce the regulation of antigen specific T-cells by TGF-β (31).

The nature of oral immune response is modifed by several factors, including characteristics of the host and the administered antigen. It must be emphasized that most of the data on development of oral tolerance is based on animal studies, and we do not know how the dose of antigen or the age of the host modifies oral tolerance in humans. Food hypersensitivities are most common in infants and in young children, and are usually spontaneously cured by the age of 2 yr, suggesting that also in humans the maturation of the gut immune system with age favors the development of oral tolerance. Little is known about the effect of antigen dose on oral tolerance in humans. In humans, the exposure to foreign food proteins, such as CM formula feeding, induces antigen-specific circulating antibodies and T-cells in infancy (17). In the infants, CM-protein specific T-cell responses seemed to decrease after continuous feeding of CM proteins, indicating the development of oral tolerance in healthy children (17). In adults, T-cell tolerance developed after prolonged ingestion of oral antigen (32). The antigen feeding did not induce a systemic humoral immune response. Interestingly, feeding antigen resulted in B-cell priming instead of tolerance, because after subcutaneous immunization higher antibody levels were detectable in the subjects who ingested the antigen compared with the non-fed subjects.

In patients with newly diagnosed diabetes, enhanced immune responses to several CM proteins have been reported (33–35). It has been suggested that these responses would be cross-reactive with islet cell antigens and participate in the autoimmune attack targeted against β-cells. The mechanisms of molecular mimicry as a cause of β-cell destruction have been suggested for several environmental antigens showing similarity with β-cell antigens. Bovine serum albumin (BSA) shares a short sequence similarity with ICA69 (33) and β-casein with glucose-transporter 4 (35). However, the evidence showing that a T-cell line specific for the environmental antigen would home to pancreatic islets and cause β-cell damage is still lacking. Alternatively, the reported hyper-reactivity to CM antigens could be considered as a failure of tolerance induction (34). When immunity to other dietary antigens has been studied in the patients with type 1 diabetes, antibodies to ovalbumin or wheat gliadin did not differ from the levels seen in healthy children (36, 37). The T-cell responses to wheat gluten were slightly enhanced, but not convincingly when compared with the enhanced T-cell responses reported to CM proteins, such as β-lactoglobulin or β-casein, in the patients with type 1 diabetes (38). If a

general failure of oral tolerance is a feature of type 1 diabetes, it is difficult to explain why immune responses to several CM proteins are increased in diabetes, whereas immune responses to other dietary proteins are nearly normal. It is possible that the enhanced immune responses to CM proteins can be considered as a marker of poor tolerance development during the first months of life, i.e., the time when CM proteins were introduced. Some data indicate that the maturation of the gut with age modifies the characteristics of generated immune response to dietary antigens (17). In humans, the permeability of the gut is higher during the first two months of life than later on (39). In the same study, Kuitunen et al. suggested that the start of CM formula may be associated with the increase of the gut permeability in infancy. Other dietary proteins than CM proteins, such as ovalbumin and wheat proteins, are started later, after the age of 5–6 months, when the maturation of the gut is more complete.

The enhanced immunity to CM proteins in newly diagnosed patients with type 1 diabetes has been suggested to be dependent on the diabetes-associated HLA-risk alleles. Enhanced T-cell reactivity to β-casein has been reported not only in patients with type 1 diabetes but also in their relatives with the same HLA-risk alleles of type 1 diabetes (40). Increased levels of antibodies to BSA were associated with the presence of HLA-DQB1*02 risk allele in children with type 1 diabetes and their non-diabetic siblings (41). Despite this, the increased levels of immunoglobulin A (IgA) and immunoglobulin G (IgG) antibodies to BSA and to β-lactoglobulin showed an association with the risk of type 1 diabetes independently of the disease-associated HLA-DQB1 alleles when the children with type 1 diabetes were compared with their HLA-DQB1-matched siblings (42). This study by Saukkonen et al. indicates that although HLA-risk genotype is associated with the immune responsiveness to CM proteins, other factors associated with the disease process itself also influence this immunity. The identification of these factors, possibly environmental, would be a matter of the utmost importance.

Dietary insulin as a trigger of insulin-specific immunity

Cow milk contains a β-cell antigen, namely insulin. The level of insulin is low in CM, being about 1–30 ng/mL in native CM and higher in colostrum (41). Insulin in CM seems to be immunogenic because development of humoral and cellular immune response has been detected in infants exposed to CM-based formula (43–45). Accordingly, the first immunization to insulin takes place in the gut by oral exposure to bovine insulin, which differs from human insulin by three amino acids. The immunogenicity of bovine insulin is based on this structural difference, which is enough to elicit an immune response in humans. Earlier when bovine insulin was used for the treatment of diabetes it induced higher levels of insulin-binding antibodies than porcine insulin, which differs from human insulin by only one amino acid (46).

The oral immunization to dietary bovine insulin in infancy does not imply the development of insulin autoantibodies (IAA), which have higher affinity than insulin-binding antibodies induced by CM exposure (44). However, this kind of priming of insulin-specific immune response may explain the epidemiological link of type 1 diabetes and CM exposure as we have suggested. Insulin has been suggested to be a primary autoantigen in the autoimmune process against β-cells, because IAA often appear as the first autoantibody in the children who develop islet cell autoimmunity (47). In animal models insulin-specific T-cells are able to transfer diabetes (48) and comprise the majority of the T-cells infiltrating the islets in NOD mice (49). During the follow-up of healthy children at genetic risk of type 1 diabetes the levels of insulin-binding antibodies increased steadily after exposure to cows' insulin in the children who developed diabetes-associated autoantibodies, whereas the levels of insulin-binding antibodies remain low in the children who did not develop autoantibodies (44). This may indicate that the development of tolerance to oral insulin is not normal in the children who show signs of autoimmunity. The question is which factors, environmental or genetic, influence the regulation of the insulin-specific immune response in early infancy? Owing to the oral route of the primary immunization to insulin, the gut immune system and factors that influence its function may play a crucial role in the development of autoimmune diabetes.

Oral autoantigen therapy in prevention of autoimmune diabetes

The role of the gut immune system in the induction of tolerance has received a lot of attention. Because oral antigen administration induces immune tolerance in animal models, oral tolerance has been studied as a treatment of harmful immune reactions towards allergens and autoantigens. Induction of oral tolerance has been used in the prevention of different autoimmune diseases including autoimmune diabetes. Feeding insulin or GAD has been reported to result in a decrease in the incidence of diabetes in NOD mice (50, 51). The incidence of diabetes decreased in female NOD mice fed with 1 mg of insulin orally twice a week for a 5-wk period from the age of 5 wk, and then weekly until 1 yr of age (50). Also, reduction of insulitis was observed. Decreased expression of IFN-γ and increased expression of IL-4,

IL-10, TGF-β have been demonstrated in the pancreatic islets of insulin-fed animals suggesting the development of a non-cytotoxic Th2 type immunity (52, 53). These studies suggest that oral insulin treatment leads to expansion of Th2-type cytokines and TGF-β producing T-cells in the pancreatic islets. Suppressed T-cell responses to GAD as well as decreased secretion of IFN-γ and increased secretion of IL-4 and IL-10 by GAD-stimulated splenocytes were observed in GAD-fed mice, too (51). These studies suggest that orally given β-cell autoantigens are able to modify the diabetes-associated autoimmunity in NOD mice.

The oral autoantigen therapy may, however, also cause harmful effects and prime autoreactive cytotoxic lymphocytes. Bergerot et al. have reported acceleration of diabetes in NOD mice by using CD8+ cells from insulin-fed mice in an adoptive transfer model (54). Blanas et al. developed a transgenic mouse model expressing ovalbumin in the β-cells under an insulin promoter (55). Oral administration of ovalbumin led to the induction of islet cell infiltrating CD8+ cells and diabetes. Also, when combined to a bacterial adjuvant, orally administered insulin exacerbated the disease in NOD mice but prevented diabetes in BB rats (56, 57). These studies clearly show that the final effect of the immunological treatments is difficult to predict and the same treatment may result in an opposite effect in different animal models. The age of exposure also seems to be critical, because oral administration of myelin basic protein to neonatal rats primes for immune responses and enhances experimental autoimmune encephalitis, whereas oral exposure in adult rats results in suppression of the disease (58). In addition, low dose or repeated administration of myelin basic protein has been shown to exacerbate the clinical course of experimental autoimmune encephalitis even in adults rats (59).

If the dysregulation of the gut immune system is associated with the development of type 1 diabetes, then oral autoantigen therapy may not be effective in the subjects at risk of autoimmune diabetes. In the worst case, it may even exacerbate the disease process. The use of adjuvants, which potentiate the Th2 switch, may be a possibility to avoid the induction of autoreactive cytotoxic cells by oral autoantigen administration. Cholera toxin B is used as an inducer of oral tolerance and Bergerot et al. used the cholera toxin B-insulin conjugate as an inhibitor of insulitis in NOD mice (60). A single oral administration of the cholera toxin B-insulin conjugate led to a decrease in the incidence of diabetes in NOD mice (60). Kolb's group also used an adjuvant together with oral and demonstrated Th2 switch in the lymphocytes infiltrating the pancreas (57). These are promising results, which encourage clinical trials using autoantigen combined with a Th2-switch inducer.

Non-specific stimulation of the gut immune system has also been shown to change the incidence of autoimmune diabetes in animal models. Cholera toxin B alone prevented diabetes in NOD mice (61) and heat-killed *Lactobacillus casei* added to the diet decreased insulitis and autoimmune diabetes in NOD mice (62). These kinds of unspecific stimuli should also be considered as triggers of autoimmune diabetes.

Gut microflora, viruses and type 1 diabetes

A prospective analysis of serum samples from siblings of children with type 1 diabetes showed that those children who developed diabetes-associated autoantibodies during the follow-up often had more serological evidence for enterovirus infections than siblings who remained autoantibody negative (63). This suggests that enterovirus infections that stimulate the gut immune system are involved with the development of β-cell autoimmunity. The mechanisms of molecular mimicry have been suggested to mediate the putative diabetogenic effect of enteroviruses. A non-structural protein, 2-C, of enterovirus Coxsackie B4 includes an area with homology to GAD (64). On the other hand, experimental studies have suggested that enterovirus could contribute to the development of autoimmune diabetes by indirect mechanisms (65). The interference with the gut immune system could provide a link between the enterovirus infections and type 1 diabetes. Viruses that replicate in the gut cause changes in the cytokine environment of the gut and thus may activate or suppress the gut-associated lymphocytes via so-called by-stander mechanisms. Viral infections may also change the permeability of the gut and thus cause alterations in the mucosal immunity to dietary proteins (66).

The role of bacterial microflora may also be important in the induction of oral tolerance. Oral ad-

Table 1. Evidence for the aberrant function of the gut immune system in patients with type 1 diabetes

Enhanced humoral and cellular immunity to food proteins, such as BSA, β-lactoglobulin, β-casein, insulin, gluten/gliadin (1, 29, 33–38,40, 41,44, 45)
Markers of immune activation in gut immunohistology (27)
Increased permeability of the gut (28)
Mucosal homing of lymphocytes derived from human diabetic pancreas (24)
Association of celiac disease with type 1 diabetes (see 1)

ministration of antigen with an invasive microbe induces systemic immunity, instead, or tolerance (67). Certain non-invasive microbes of the normal gut flora have been used in the manipulation of the gut immune system, such as *Lactobacillus* GG. *Lactobacillus* GG has been used in clinical studies on the treatment of food allergy (68). The mechanisms of the *Lactobacillus* GG treatment are not fully understood. It has been suggested that probiotic bacteria may promote endogenous barrier mechanisms in patients with food allergy and decrease intestinal inflammation (68).

Summary and conclusions

The gut immune system is a major T-cell organ in humans. It has a key role in controlling the immune responsiveness to food and microbial antigens encountered via the gut (Fig. 1). Several studies indicate that an aberrant function of the gut immune system is found in type 1 diabetes (see Table 1). In epidemiological studies, exposure to dietary CM proteins have been associated with the risk of type 1 diabetes suggesting that the immune system of the individuals susceptible to type 1 diabetes could not handle these proteins normally. Several experimental studies have shown that dietary factors influence the characteristics of islets infiltrating lymphocytes and, finally, the development of autoimmune diabetes (Fig. 2). The common lymphocyte homing properties in the gut and the pancreas provide an immunological link between these two organs. Accordingly, the environment of the gut immune system may affect the lymphocytes, which infiltrate the pancreas. The evidence showing that the

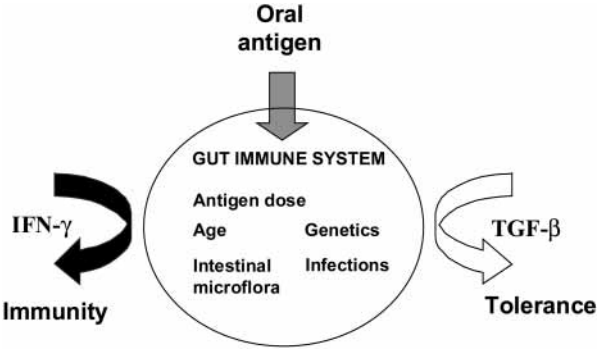

Fig. 1. The nature of the immune response to oral antigen is determined by several factors, which interfere with the function of the gut immune system, such as genetic background of the host, intestinal microflora, gastrointestinal infections, and the cytokine environment of the gut. Immune response to dietary antigen is controlled by mechanisms of oral tolerance in healthy individuals. In the gut immune system, the production of TGF-β has been associated with the development of immune tolerance whereas high production IFN-γ has been associated with the induction of immunity to ingested antigens. In autoimmune diabetes, the balance between immunity and tolerance in the gut immune system has been suggested to be disturbed.

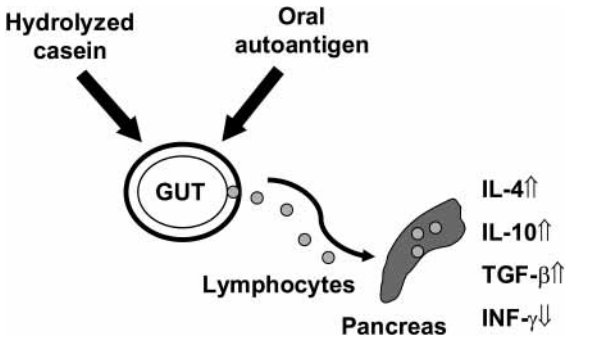

Fig. 2. Diet affects the cytokine profile of islet-infiltrating lymphocytes. Diet of hydrolyzed casein after weaning or orally administered autoantigens, such as insulin and GAD, have been reported to decrease the incidence of autoimmune diabetes in animal models. Both of these treatments induce a Th2-type cytokine switch in the lymphocytes infiltrating the pancreatic islets, supporting the view that an immunological link exists between the gut and pancreas.

priming of insulin-specific immune response occurs in the gut by dietary insulin may be the connection between the gut immune system and β-cell autoimmunity. These observations provide ground for the attempts to find a cure for type 1 diabetes by means that affect the function of the gut immune system.

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