Long-Term Effects of Weaning Habits: Type-1 Diabetes

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Introduction

Type-1 diabetes is an autoimmune disease which attacks insulin-producing β cells in the pancreas. This autoimmune process is characterized by the appearance of circulating autoantibodies against β-cell antigens, such as insulin, glutamate decarboxylase (GAD) and tyrosine phosphatase. The infants of mothers with type-1 diabetes do not have autoimmune diabetes despite the transfer of IgG antibodies, including autoantibodies to β cells, to the child via the placenta. This indicates that autoantibodies against β-cell antigens, although being markers of β-cell inflammation, do not transfer the disease. Also in animal models of type-1 diabetes the evidence indicates that autoimmune diabetes is mediated by autoreactive T cells which infiltrate the islets and mediate the destruction of β cells.

The genes of the HLA complex, among them especially the DQ genes, are most important in type-1 diabetes susceptibility. HLA DQ2 and/or DQ8 alleles are found in about 90% of children with type-1 diabetes. On the other hand, the genotype of disease susceptibility is found in about 22% of the population, showing the impact for environmental factors in the modification of disease development. We have seen a rapid increase in the incidence of type-1 diabetes during the last decades, in particular in young children [1]. This kind of change in the incidence cannot be totally explained by genetic changes and thus it is obvious that environmental factors modify the incidence of type-1 diabetes. Dietary factors, especially during the first year of life, have been intensively studied as putative environmental risk factors for β-cell autoimmunity and type-1 diabetes.

Accumulating data suggest that the gut immune system plays a key role in the development of type-1 diabetes, supporting the view that dietary factors
are able modify the risk of type-1 diabetes. In non-obese diabetic (NOD) mice, islet-infiltrating lymphocytes express α4β7 integrin, a homing receptor of the gut mucosa, and antibodies blocking this receptor prevent diabetes [2]. Mesenteric lymphocytes from young non-diabetic NOD mice include diabetogenic lymphocytes which are able to transfer diabetes to NOD/scid mice [3]. In humans, some reports suggest that autoreactive T cells may originate from the gut immune system. T cells derived from the pancreas of a patient with type-1 diabetes adhered to the mucosal and pancreatic endothelium [4]. GAD-reactive T cells from patients with type-1 diabetes expressed gut-associated homing receptor α4β7 integrin [5]. As markers of intestinal immune activation, increased expression of HLA class-II antigen, α4β7 integrin, IL-4 and IL-1β has been found in the intestinal mucosa of children with type-1 diabetes without associated celiac disease (CD) [6]. Several studies suggest that patients with type-1 diabetes, especially if diagnosed at an early age, have failure of oral tolerance manifested with increased immunity to dietary cow’s milk (CM) proteins [7] as well as to wheat gluten [8]. Aberrancies of the gut immune system in children prone to type-1 diabetes may at least partly explain the harmful effects of diet in these individuals, whereas the same dietary factors may be innocent in the majority of children.

**Weaning to Hydrolyzed Cow’s Milk Formula as Prevention of Type-1 Diabetes**

Dietary factors, especially when modified during the weaning period, affect the development of autoimmune diabetes in animal models of type-1 diabetes: biobreeding (BB) rats and NOD mice. In these animal models, autoimmune diabetes develops spontaneously, and there is no direct evidence showing that any putative dietary factor(s) could directly cause the development of destructive insulitis and autoimmune diabetes. Weaning to a diet of hydrolyzed CM formula [9, 10] protects from autoimmune diabetes in both the above-mentioned animal models. A diet of hydrolyzed protein induces functional changes in the islets infiltrating lymphocytes [10], which indicates that an immunological link between diet and autoimmune diabetes exists. Studies in the NOD mouse model suggest that weaning to casein hydrolysate induces regulatory T cells which are responsible for the prevention of autoimmune diabetes [11]. Casein hydrolysate, when given either continuously or for 10 weeks after weaning, was highly effective in preventing autoimmune diabetes (32-week incidence 4.6 vs. 58.8%) in NOD mice. Spleen cells from protected NOD mice failed to adoptively transfer diabetes into irradiated syngeneic recipients. Furthermore, splenocytes from diet-protected mice inhibited adoptive diabetes transfer with splenocytes from diabetic donors (incidence 50 vs. 94%, p < 0.001). The animal studies thus suggest that weaning to a hydrolyzed
diet instead of weaning to a diet containing whole proteins is protective from type-1 diabetes, but do not directly suggest a diabetogenic role for CM. Epidemiological studies, however, propose dietary CM proteins as a putative trigger of type-1 diabetes by showing a correlation between the consumption of CM products and the incidence of type-1 diabetes in several countries [12]. Also the exposure to CM proteins during early life has been reported to imply an about 1.5- to 2-fold risk of type-1 diabetes in children who were breastfed for <3–4 months or exposed to CM formula before the age of 3–4 months [13–16]. A Swedish study indicated that early exposure to CM proteins was a risk factor for type-1 diabetes diagnosed at an early age [16]. When the effect of the HLA risk genotype was considered, the risk of diabetes related to early CM exposure was more pronounced in those individuals who had the ‘high-risk’ genotype of disease [15]. An association between early CM exposure and the risk of type-1 diabetes has not been observed in all epidemiological studies performed, but according to two meta-analyses of case-control studies, the overall risk of type-1 diabetes was slightly increased being about 1.5-fold when the child was exposed to CM proteins before 3 months of age [17, 18]. Most of the studies are based on retrospectively collected infant diet data and in some studies the participation rate of controls was lower than that of the cases, factors which may have biased the results of these studies [17]. Thus, prospective studies are needed to confirm the possible link between CM exposure and type-1 diabetes. Recent reports from the DAISY study and the German BABYDIAB study did not show an association between CM exposure and β-cell autoimmunity [19, 20]. The definition of CM exposure may differ between different studies; for instance in the German study all CM-containing products (also hydrolyzed formula that has been suggested to be protective) were recorded, and thus the results are not comparable to studies in which exposure to CM formula containing whole CM proteins was indicated as a risk factor, e.g. in a Finnish birth-cohort study [21]. Since the follow-up time in all these studies was relatively short and the number of children who developed type-1 diabetes was limited, the association of CM exposure with the emergence of β-cell autoimmunity, and not with the risk of type-1 diabetes, was reported. In the Australian BABYDIAB study of children from families with type-1 diabetes, no association of CM formula exposure and the appearance of β-cell autoantibodies was demonstrated [22]. Instead, in a Finnish birth-cohort study, the Diabetes Prediction and Prevention study, exposure to CM formula before 4 months of age implied an about 5-fold risk for the development of multiple autoantibodies and especially autoantibodies against tyrosine phosphatase in children who all carried the HLA DQB1*0302 risk allele of type-1 diabetes [21]. The methodological problems in the follow-up studies of dietary exposures are obvious, and thus randomized controlled intervention studies are needed to answer the question of the protective effect of CM avoidance during the first months of life or weaning to hydrolyzed formula.
The international Trial to Reduce IDDM in Individuals at Genetic Risk study was planned to study the question whether elimination of CM proteins in the diet and the use of hydrolyzed formula during the first 6 months of life could be protective from type-1 diabetes [23]. By 24 months of age, 1/53 (1.9%) of the subjects in the casein hydrolysate group had developed autoantibodies, whereas 6/48 (12.5%) in the control group had done so (p = 0.036, Fisher's exact test) [23]. By the age of 5–7 years life-table analysis, showed, after adjustment for duration of study formula feeding, a significant protection by the intervention from positivity for ICA (p = 0.02) and at least one autoantibody (p = 0.03) [24].

In some epidemiological studies not only early exposure to CM proteins, but a high intake of CM proteins later in life implied a risk of type-1 diabetes [14, 25]. This was also evident in a prospective follow-up study of the healthy siblings of Finnish patients with type-1 diabetes [25]. These studies suggest that CM could contain a diabetogenic factor which contributes to the development of type-1 diabetes.

Several candidates for a diabetogenic factor in CM have been suggested, such as bovine serum albumin, β-casein and CM insulin. Bovine insulin in CM induces primary immunization to insulin in infants exposed to CM formulas [26, 27]. According to a hypothesis, weaning to CM results in immunization to bovine insulin, and if this happens during the time when gut maturation is incomplete, an aberrant immune response to insulin may be developed. Later this immune response to dietary insulin could be activated against insulin-producing β cells [7].

In our follow-up study of children at genetic risk of type-1 diabetes, the levels of bovine insulin-binding antibodies increased after the primary immunization (i.e. exposure to CM) in children who later developed β-cell autoimmunity, whereas in autoantibody-negative children the insulin-binding antibodies remained at lower levels [28]. This may indicate a dysregulation of oral tolerance in children prone to β-cell autoimmunity. Indeed, subclinical intestinal immune activation has been associated with type-1 diabetes [6, 29] and could favor the development of a harmful immune response to insulin.

The weaning to CM during early infancy may also influence the development of autoimmune diabetes by nonspecific mechanisms. The introduction of CM proteins to the infant’s diet is a strong immunological stimulation manifested as the development of antigen-specific humoral and T-cell responses to foreign CM proteins [30] and induction of soluble adhesion molecules [31]. Thus, early stimulation of the gut-associated immune system by foreign proteins may prime the gut immune system and further activate β-cell autoimmunity by nonspecific bystander mechanisms. It is possible that a diet containing hydrolyzed proteins, i.e. less immunogenic peptides, instead of CM proteins prevents autoimmune diabetes due to less stimulation of the intestinal immune system during the first months of life.
Wheat Gluten as a Trigger of Type-1 Diabetes

CD is more common in patients with type-1 diabetes than in the general population; the prevalence varies between 5 and 8% in studies of patients with type-1 diabetes in different populations [32]. The increased prevalence is at least partly explained by the shared HLA-risk genotype, DQ2 and DQ8.

Studies in animal models suggest that wheat gluten could trigger autoimmune diabetes. Wheat gluten when added to the diet of BB rats causes an increased incidence of autoimmune diabetes suggesting that dietary wheat gluten could contribute to the development of autoimmune diabetes [33]. A gluten-free diet prevents autoimmune diabetes in NOD mice [34]. In NOD mice a wheat-based diet induced a Th1 response in the gut [35].

Weaning to gluten-containing food before 4 months of age has been suggested to increase the risk of β-cell autoimmunity in the German BABYDIAB study [19]. This is an interesting finding, but should be interpreted carefully since it is based on a small number of individuals who developed β-cell autoantibodies. In the North American DAISY study both early (i.e. before 3 months of age) and late introduction of gluten-containing cereals after 7 months of age implied a risk of β-cell autoimmunity [20]. The authors suggest that there may be a window of exposure to cereals in infancy outside which initial exposure increases the risk of β-cell autoimmunity in susceptible children.

Some studies suggest that dietary wheat gluten may trigger impaired β-cell function and could accordingly be a candidate to trigger autoimmune diabetes also later in life [36]. Interestingly, in a trial of 17 autoantibody-positive healthy first-degree relatives of diabetic patients, the insulin response to intravenous glucose improved after 6 months of a gluten-free diet. Autoantibody titers did not show significant changes. After returning to the normal diet the acute insulin response decreased in 10 of 13 subjects during the following 6-month period. These findings indicate that 6 months of gluten elimination does not influence humoral autoimmunity, but may have a beneficial effect on the preservation of β-cell function in subjects at risk of type-1 diabetes [36].

The possible diabetogenic mechanisms of wheat gluten are not known, and studies of the mechanisms are few. It is possible that wheat gluten triggers autoimmunity and/or inflammatory changes in individuals who carry the HLA risk genotype common to CD and type-1 diabetes. This view is supported by Italian studies reporting that in vivo and in vitro stimulation with gliadin results in an enhanced local intestinal immune response in patients with type-1 diabetes [29, 37]. This inflammatory response to challenge was found in diabetic patients with normal CD serology (anti-gliadin antibody, anti-endomysial antibody, and anti-tissue transglutaminase antibodies) and a morphologically normal jejunal mucosa. It is possible that gluten-induced inflammatory changes favor the activation of β-cell autoimmunity in the gut immune system without any specific cross-reactive autoimmunity between...
wheat gluten and β-cell antigens. However, recently antibodies to wheat storage protein were demonstrated in patients with type-1 diabetes but not in control individuals [38]. The reactivity to the gluten-derived protein was associated with β-cell damage in BB rats, and the authors suggest that immunity to wheat gluten may be involved with the pathogenesis of autoimmune diabetes. It is evident that more clinical and mechanical studies are needed to clarify the possible role of wheat gluten in β-cell autoimmunity and type-1 diabetes.

Low Vitamin-D Intake and Type-1 Diabetes

A large multicenter trial covering 7 centers in Europe and comprising data on vitamin-D supplementation to 820 patients with type-1 diabetes and 2,335 controls showed a protective effect (odds ratio 0.67) of vitamin-D supplementation in infancy [39]. In a Finnish birth-cohort study including children born in 1966 in northern Finland, a low vitamin-D intake during the first year of life implied an increased risk of type-1 diabetes [40]. Children who regularly took the recommended dose of vitamin D (2,000 IU daily) had a relative risk of 0.22 compared with those who regularly received less than the recommended amount. Children suspected of having rickets during the first year of life had a relative risk of 3.0 compared with those without such a suspicion. It is difficult to estimated whether high doses of vitamin D or its analogs could be effective in the prevention of human type-1 diabetes in the general population in whom vitamin-D supplementation was followed.

The mechanisms of vitamin D in the development of autoimmune diabetes were studied in animal models, but in these studies high doses of 1,25(OH)2D3 were administered and not vitamin D. The protective mechanisms of 1,25(OH)2D3 or its analogs in the NOD mouse model were suggested to be mediated by increased apoptosis of Th1 cells, induction of skew from Th1 to Th2 immune response, and activation or induction of regulatory CD25 high CD4 cells [41].

References

Pancreatic autoimmunity at birth could represent a "seed" for later disease development. Variants of the HLA-DQB1 gene, which are associated with Type-1 diabetes, are present in infant immune cells derived from intrauterine life.

The following are key points from the references:

- Glutamate decarboxylase-reactive peripheral blood lymphocytes from patients with IDDM express gut-specific homing receptor alpha4beta7-integrin. Diabetes 1997;46:583–588.
- Potential mechanisms by which certain foods promote or inhibit the development of spontaneous diabetes in BB rats: Dose, timing, early effect on islet area, and switch in infiltrate from Th1 to Th2 cells. Diabetes 1997;46:589–598.
- Emergence of diabetes associated autoantibodies in the nutritional prevention of IDDM (TRIGR) project. Diabetes 1999;48(suppl 1):A45.

**Discussion**

_Dr. Paerregaard:_ You mentioned that type-1 diabetes is a T-cell-mediated disease and also that in animal models you could transfer T cells and the disease. We know that lymphocytes are present in breast milk and that by ingestion of breast milk the infants also introduce maternal T cells into their gut and that these T cells interact with the immature immune system of the infants. We also know that in certain respects this can have consequences later, for instance if the infant needs an organ transplantation at a later stage, it may influence whether this organ is rejected or not. Could ingestion of T cells from diabetic mothers be a risk factor for the infants? I know that breast milk in general probably promotes protection against the type-2 diabetes, but in the offspring of diabetic mothers could these maternal T cells actually be a risk factor for type-1 diabetes?

_Dr. Vaarala:_ First, I think that we have actually not studied breast milk enough in this context. We know that breast milk contains cytokines, growth factors and immune cells. We also know that the cytokines, for example breast milk transforming growth factor-β, modulate the development of at least the antibody responses in children [1], so this is definitely a very interesting area of study. If we think about breast milk from diabetic mothers, whether it is protective or not, we have some epidemiological data that actually show very clearly that the risk of developing type-1 diabetes is decreased in the offspring of mothers with diabetes when compared to the offspring of fathers with diabetes [2]. There is no explanation for this, but something occurs there during pregnancy or the first months of life, and maternal diabetes actually protects from the genetic risk load. Whether it is mediated by immune cells against β-cell antigens present in the breast milk, that is an interesting hypothesis, but the effect is the opposite to what you suggested.
Dr. Exl-Preysch: Could you comment or speculate on that huge epidemiological study published last year in Germany in which a group of children with newly diagnosed atopic eczema was compared with paired controls, and the incidence of type-1 diabetes was looked at [3]. The group with eczema had a significantly lower incidence of type-1 diabetes than the group without eczema. They speculated on the Th1/Th2 ratio, and stated that when these children have a Th2 increase they have allergy but not diabetes and also the other way round. I would like to have your opinion on that.

Dr. Vaarala: When we think about Th1/Th2 polarization, the next idea of course is to think that if children with atopic diseases have a type-2 response there is downregulation of the type-1 response that is associated with diabetes. Thus you don't see these two diseases in the same individuals. I think that this is the hypothesis behind this kind of study. Now there are several studies on the association of atopic asthma and atopic diseases with type-1 diabetes [4], and the studies are quite discrepant. There are even studies showing that atopic asthma is actually associated with type-1 diabetes [5], so I think that this issue is yet not clear enough and personally I do not believe too much in this simple type of explanation based on type-1/type-2 polarization for the epidemiology of allergic and autoimmune diseases. Th1/Th2 polarization may be the factor in the effect on antigen-specific T cells. But it is much more likely that T-regulatory cells are more important in the context of disease development. They control both type-1 and type-2 immune responses and similar kinds of environmental factors, for example microbial load that regulates the induction of regulatory cells may be a risk factor for both allergies and type-1 diabetes, the incidence of these diseases is increasing at the same time. So I would think that the answer will be found in the regulatory mechanisms, and the genetic background of the children will determine whether they get type-1 diabetes or another type of immune-mediated disease such as atopic allergy.

Mr. Benyacoub: Coming back to this aspect of a potential epigenetic effect, is anyone investigating the methylation profile of these children, particularly with regard to the 2 or 10% of them who developed type-1 diabetes?

Dr. Vaarala: When I was listening to the previous presentation I got the idea that this should be studied now, especially the effect of dietary factors on T cells and immune responses, the development of T cells.

Dr. H. Hoekstra: The clinical association between type-1 diabetes and celiac disease is mostly that first diabetes and then celiac disease are diagnosed. Is this not a point against your theory?

Dr. Vaarala: I must say that I don't understand how would it be against my theory, because I think it supports my theory. If you think that triggers of the intestinal immune system are associated with type-1 diabetes, the presence of these triggers will lead to the development of type-1 diabetes, and the same factor is actually affecting the development of celiac disease very near the diagnosis of type-1 diabetes. So you don't need to wait 10 years, they occur together just because of this association with the trigger.

Dr. H. Hoekstra: The clinical presentations of classical celiac disease and diabetes are mostly quite obvious. So if celiac disease is an important factor to de-clench diabetes, wouldn't you expect that celiac disease will present itself earlier than diabetes?

Dr. Vaarala: Actually my point is that exposure to gluten and an aberrant immune response to gluten is associated with type-1 diabetes. It could play a role in triggering type-1 diabetes, but celiac disease as a trigger of type-1 diabetes, that was not my theory. The children who get type-1 diabetes do not have or quite seldom have celiac disease. They have celiac disease because they share the same genetic background, so the incidence of celiac disease is higher in patients with type-1 diabetes because of this accumulation of the celiac disease-risk genotype. But the occurrence of celiac disease is not higher than that estimated from the HLA-risk genotype when compared to the population. But the response to gluten may be aberrant because of this binding
ability of gluten peptides by HLA molecules and triggering of T cells. But the process
stops, there is sub-clinical inflammation in the intestinal immune system and this does
not necessarily lead to a celiac disease.

**Dr. Bee Wah Lee:** Have there been any trials on probiotics preventing type-1
diabetes when given very early in life or in experiments on animal models?

**Dr. Vaarala:** There is one animal study from Japan in which lactobacillus GG was
given to NOD mice and it resulted in the prevention of autoimmune diabetes [6]. We
started a study 2 years ago in which probiotics, a combination of 4 different probiotics
or bacteria, were given to children with a genetic risk of type-1 diabetes. We have seen
immunological changes induced by this preparation but, of course, we have no data to
show whether it has any effect on β-cell autoimmunity. But we hope to be able to bring
this study to an end and then have some information.

**Dr. Fritsché:** You mentioned in your talk that you have identified the hydrolyzed pep-
tide. Can you tell us a little bit more about its function in deviating to the Th2 pathway?

**Dr. Vaarala:** This evidence comes from animal models and I still do not have any
human data on this. Evidence is based on a feeding protocol: the mice and rats that
received hydrolyzed peptides at weaning have this kind of type-2 immune response
phenotype in the islets when compared to animals receiving normal proteins [7]. So we
have not actually identified a single peptide in hydrolyzed formula that drives this kind
of development, and we still do not have evidence from human studies, but this will be
studied in the TRIGR and the FINDIA studies.

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