Celiac Disease: Effect of Weaning on Disease Risk

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Introduction

From the weaning period and onwards the intestinal mucosa is exposed to an increasing number of antigens, e.g. food components and microorganisms. Of all the antigens that reach the systemic circulation from the gut lumen, only a minority are potentially harmful to humans and need to be defended against. The majority of intestinal antigens do not require a protective immune response, but may even be beneficial for the individual. Thus, the mucosal immune system must have the capacity to discriminate between when an appropriate protective immune response to harmful foreign antigens is required and when a muted or non-response is preferable. One important component of the latter is oral tolerance, which may be defined as a systemic hypo-responsiveness or non-responsiveness of mature T and B lymphocytes to antigen challenge after prior oral exposure to the antigen [1, 2].

Celiac disease (CD), or permanent gluten-sensitive enteropathy, develops because tolerance to ingested wheat gluten (gliadins and glutenins), and related proteins from rye and barley never develops, or is broken after it has developed. The disease is characterized by inflammation of the small intestine resulting in crypt hyperplasia, villous atrophy and flattening of the mucosa. Other characteristics are an increased number of intraepithelial lymphocytes (IELs) and lamina propria lymphocytes, increased serum concentrations of IgA antibodies towards gliadin and the autoantigen tissue transglutaminase. When gluten is withdrawn from the diet the mucosal
morphology is restored, the specific antibody levels become normal, and symptoms of the disease disappear [3].

CD is an acquired disorder, which can be diagnosed in early childhood with classical symptoms such as diarrhea, malabsorption and failure to thrive, but also later in life, even in adults who show a wider and more diffuse spectrum of symptoms. Poor diet compliance and undiagnosed disease, which is frequent in adult populations [4], are associated with increased morbidity and mortality [5].

**A Multifactorial Etiology**

CD is a chronic inflammatory disease. Collectively, these diseases have multifactorial etiologies. The genetic component is strong in CD with a high sibling relative risk and high concordance between monozygotic twins (75%) [3, 6]. The recent Swedish epidemic of CD with classical symptoms in children below 2 years of age strongly supports an etiological role of environmental factors, such as early infant feeding practices (fig. 1) [7, 8]. Thus, while it is likely that environmental components trigger the disease in genetically predisposed individuals, the complex interplay between genetic and environmental factors makes it difficult to delineate the complex multifactorial pattern. Nonetheless, CD is the best understood HLA-linked disease.

CD is a particularly useful model for increasing our understanding of the complex immunological diseases [9]. Exposure to gluten, or certain known peptides thereof, is a prerequisite for CD development, and once established the disease can be turned on and shut off by introducing or withdrawing gluten from the diet. CD is a polygenic disease, and most of the genes involved are still unknown [6]. However, over 90% of the patients express the MHC class II molecule HLA-DQ2 and the remainder usually HLA-DQ8, both of which predispose for the disease [10].

Finally, simple access to the affected organ – the intestinal mucosa – by endoscopic or capsule biopsies allows detailed ex vivo studies such as isolation and characterization of disease-relevant cell populations. Such studies have shown that CD is associated with an abnormal T-cell-initiated immune response to gluten, but the detailed pathogenesis still remains to be elucidated. The link between environmental factors besides gluten and the development of CD also needs to be explored further.

In the mid 1980s CD became the most common chronic disease in Swedish children after IgE-mediated allergy (fig. 1) [7, 8]. The epidemic pattern is unique for a chronic disease, strongly suggesting that environmental factors contribute substantially to precipitation of the disease and, thus, prevention should be possible. A recent incident case-referent study contributed to the identification of some of these causal environmental risk factors [11], which are discussed below (fig. 2).
Fig. 1. The Swedish epidemic of CD. From Ivarsson [8] with permission.

Fig. 2. A crude model on the effect of weaning on CD risk.

**Amount of Dietary Gluten: An Important Causal Factor**

The dose of dietary antigen ingested may influence whether or not oral tolerance develops [12]. This is easily demonstrated in experimental animals, and is possibly true for humans as well. However, whether this dose effect also applies to gluten in relation to the risk of developing CD is not known.

Healthy Swedish and Italian infants were reported to have a larger consumption of wheat gluten than infants from Finland and Denmark [13, 14] and, interestingly, the former countries also reported a higher occurrence of CD. The study design did not enable adjustment for differences in other potentially causal exposures. Moreover, a Swedish case-referent study [15] reported that CD cases, more often than referents, were introduced to gluten by means of gluten-containing milk cereal drinks (MCDs), which, by Swedish tradition, are used from 6 months of age rather than follow-on formulas.
Although the design did not allow estimation of the amount of gluten consumed by the infants, this study still suggests that the amount of gluten is a causal factor, as bottle-feeding more readily contributes a larger amount of food as compared to feeding by cup or spoon.

We recently reported results from an incident case-referent study of 491 cases and 781 referents, which was population-based and had a high participation rate [11]. Hence, the results should be representative for Sweden at large and also valid for children in general. As only incident cases, i.e. newly diagnosed ones, were included, the recall period was comparatively short which reduced the risk of recall bias. A comprehensive questionnaire, which did not reveal our focus on CD, concerning the children’s diet and health in general was mailed to the families. We used a semi-quantitative food frequency questionnaire to assess the consumption of gluten-containing cereals. Multivariate analyses were used to adjust risk estimates for confounding and to suggest causal relationships. For the first time the design of the study allowed assessment of the consumption of gluten-containing cereals on an individual level [11]. The amount of gluten-containing flour consumed during introduction was assessed by the single food item which contributed the largest amount during the first 2 weeks of consumption, while the amount at 7 months of age was based on all food items consumed at that age. Introduction of gluten-containing foods in large amounts, as compared to small or medium amounts, was an independent risk factor for CD development (adjusted odds ratio (OR) = 1.5: 95% confidence interval (CI) 1.1–2.1). By use of multivariate analyses, differences in breastfeeding practices and the age of the infant when first introduced to gluten-containing foods could be adjusted for. Moreover, the type of food used as the source of gluten, i.e. solid foods or MCDs, was not a significant independent risk factor. Thus, there are reasons to argue that our observations are applicable not only to Sweden, with its traditional use of MCDs. These results strongly suggest that the introduction of gluten in larger amounts increases the risk of CD [11].

Our ecological study of the Swedish epidemic, in which we used aggregated data to explore any temporal relationship between changes in the incidence rate and in infant dietary patterns, supports the theory that the quantity of gluten consumed during infancy is a risk factor for CD. The rise in incidence was preceded by a twofold increase in the average daily consumption of gluten estimated by the use of MCDs, and later the decline in incidence coincided with a consumption decrease by one third [7].

Gluten-sensitized individuals do respond in a time-related and dose-dependent fashion to gliadin [16]. Recently an explanation for the HLA-DQ2 gene dose effect for the development of CD was proposed. Individuals homozygous for the HLA-DQ2.5 molecule on their antigen-presenting cells present a broader range of gliadin peptides to T cells than HLA-DQ 2.5/2.2 heterozygous individuals, while HLA-DQ 2.5/non-DQ2 heterozygous individuals are poor presenters and have only a slightly increased risk of developing CD.
These results and others are indicative of a quantitative model for disease development. However, it is still not settled whether gluten as a risk factor for CD acts in a dose-dependent manner or whether there is a threshold effect. If the latter is true it is likely that the amount of gluten required to pass the threshold is lower in HLA-DQ2.5 homozygous compared to heterozygous individuals.

Thus, there is evidence to suggest that during infancy consumption of a large amount of gluten-containing flour, which increases the antigen dose, increases the risk of CD (fig. 2). However, the amount of gluten tolerated may be modulated not only by the genetic predisposition of the individual but also by environmental exposures besides gluten.

**Does Age at Introduction Matter?**

It is possible that there is an age interval during which humans have decreased ability to develop oral tolerance to a newly introduced dietary antigen [12]. Hypothetically, the age of the infant at introduction of gluten into the diet might influence the risk for CD.

Although, previous comparisons of English CD patients suggested that earlier introduction of dietary gluten resulted in earlier presentation of the disease, no such relationship was found in later studies taking differences in breastfeeding duration into account [18]. On the other hand, a delayed introduction of gluten into the diet of infants was suggested to contribute to the decline in CD incidence in the United Kingdom in the 1970s [19, 20]. In contrast, comparable dietary changes occurred in Sweden without any observed change in incidence. Furthermore, the increased incidence in Swedish children in the middle of the 1980s was preceded by a postponed introduction of dietary gluten from 4 to 6 months of age [7]. Obviously, these ecological observations are contradictory. However, a study design based on aggregated data cannot by itself provide conclusive evidence.

Case-referent studies based on individual data enable adjustments for differences in other potentially causal exposures. After having adjusted for differences in breastfeeding duration, such studies suggested that the age of the infant at introduction of dietary gluten was not a causal factor with respect to CD risk [15, 21, 22]. In our incident case-referent study we found a bivariate association indicating an increased risk for CD when dietary gluten was introduced within the age interval of 5–6 months. However, this association no longer remained statistically significant when adjusting for differences in dose of gluten given during introduction and breastfeeding variables [11].

CD and diabetes mellitus type 1 are interlinked, i.e. suffering from one of them increases the risk for the other. Recently, two cohort studies on children with increased risk for diabetes mellitus type 1 investigated the
association between the age at introduction of dietary gluten and indicators of autoimmunity. One of them suggested that it is beneficial to introduce gluten within an interval of 4–6 months of age as compared to both earlier and later introduction [23], while the other study did not find that age at introduction influenced the risk of autoimmunity [24]. Both studies considered differences in breastfeeding duration in the analyses, but not the amount of dietary gluten given during the introduction which consequently remains a potential confounder.

Thus, whether or not the infant’s age at the time of gluten introduction is a risk factor for CD has not been settled. Most studies refute it as an independent risk factor, but it should remain on the agenda for further exploration.

**Infections and Innate Immunity**

In the 1980s, Kagnoff et al. [25] suggested that gastrointestinal infection with adenovirus type 12 could initiate CD because of significant sequence similarities between the E1b protein produced by this virus and A-gliadin of gluten, a hypothesis later questioned but not yet excluded as a possibility. Furthermore, gastrointestinal infections cause a disruption in the barrier function of the small intestinal mucosa, which theoretically could result in an increased antigen penetration and unfavorable immune response. We found that, compared to referents, CD cases were more often born in the summer [26], and therefore more often introduced to gluten during the winter when infections are more frequent. Thus, it is conceivable that the Swedish epidemic was partly caused by a change in the infectious panorama, or an interaction between infant feeding and infections.

Interestingly, we recently discovered that rod-shaped bacteria are frequently associated with the intestinal mucosa of CD patients, both with

**Fig. 3.** Presence of rod-shaped bacteria in jejunal biopsies from a CD patient with active disease. Scanning electron micrographs of jejunal biopsies from 1 control patient (a, c, e) and 1 CD patient with active disease (b, d, f). a Normal villus architecture with leaf-shaped villi. b Totally flat mucosa corresponding to subtotal villous atrophy. c No bacteria and normal cell appearance with uniform enterocytes, showing regularity both in shape and size. d Presence of large numbers of bacteria and disturbed cell structure with cobblestone appearance, irregularity in shape and size of the enterocytes. e Normal ultrastructure of the apical surfaces of enterocytes with thick glycocalyx, completely covering the microvilli. f Presence of bacteria in bouquet-like groups and severe distortion of ultrastructure of the apical surfaces of the enterocytes showing prominent decrease in glycocalyx thickness and irregularly oriented and barely visible microvilli. g Frequency of jejunal biopsies from CD patients and controls with adherent bacteria. n = Number of biopsies analyzed. Statistically significant differences as determined by the $\chi^2$ method are indicated. a, b Bars correspond to 300 $\mu$m. c, d Bars correspond to 30 $\mu$m. e, f Bars correspond to 3 $\mu$m. From Forsberg et al. [27] with permission.
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active and inactive disease, but not with that of controls as revealed by scanning electron microscopy (fig. 3) [27]. Moreover, the presence of bacteria is associated with a particular lectin-staining pattern of the intestinal mucosa. It seems therefore that unique carbohydrate structures of the glycocalyx/mucous layer are likely discriminating features of CD patients. These glycosylation differences could facilitate bacterial adhesion. Adhesion/infection by these yet undefined bacteria could precipitate disease in genetically susceptible individuals. Alternatively, bacterial adhesion may precipitate a change in the glycosylation pattern. We have also shown that there is a strong IEL response in CD with a highly significant increased expression of the cytokines interferon (IFN)-γ and interleukin (IL)-10, without a concomitant increase in the expression of tumor necrosis factor-α or transforming growth factor-β, and with marked shift of the IFN-γ and IL-10 production from the lamina propria to the epithelium. This may cause both recruitment of IELs and a leaky epithelium. Hence, the epithelial reaction may be critical for disease development [28]. This view was recently supported by the observation that enterocyte expression of the MICA protein, a non-conventional HLA class-I molecule, is upregulated in patients with active CD [29, 30]. The expression can be induced by stress [31], but as now shown also by gliadin, or peptides thereof [29], and by IL-15 [29, 30]. MICA serves as ligand for the activating NKG2D receptor expressed at the surface of NK cells, and some CD8 α/β T and γ/δ T cells. Hüe et al. [29] also showed that NKG2D expressing lymphocytes can lyse epithelial cells, which may explain the villous atrophy typical for CD. It seems that IL-15 is a key player in these events by upregulating both MICA and NKG2D [29, 30]. The latter has been shown to play a key role in other immune-mediated disorders. It may exert beneficial functions during infections but could also serve as an immune activator that can tip the balance in favor of autoimmunity and chronic inflammation [32]. Whether the rod-shaped bacteria associated to the mucosa in CD patients stress the epithelial cell to increased expression of MICA is not yet known.

We have also found that the expression of mucin-2, α-defensins HD5 and HD6, and lysozyme is increased in active CD but returns to normal in treated CD. Their expression levels correlated to the IFN-γ mRNA levels of IELs, suggesting that this cytokine upregulates the expression of these molecules. Metaplastic Paneth cells were seen in the small intestine of children with active CD. These cells may at least partly be responsible for the increased levels of α-defensins and lysozyme [27]. Recently, Maiuri et al. [33], incubating small intestinal biopsies in vitro, gave further evidence of the involvement of innate immunity in CD. They showed that the gliadin fragment can activate this immune system, affecting the in situ T-cell recognition of dominant gliadin epitopes. IL-15 also seemed to be involved in this activation [33].

In our incident case-referent study [11] we found that children who experienced three or more infectious episodes before 6 months of age had an
increased risk of CD before 2 years of age (adjusted OR = 1.4, 95% CI 1.0–1.9; Ivarsson et al., to be published). This was true even when episodes of gastroenteritis were excluded, and after adjustments for differences in infant feeding patterns. Interestingly the risk of CD increased considerably if, in addition to having many infections, the child was also introduced to gluten in large amounts as compared to small and medium amounts.

Thus, it is possible that common infections, both gastroenteritis and other types of infections, play a causal role in the development of CD (fig. 2). This can be due to the fact that infectious episodes might increase gut permeability followed by increased antigen penetration and activation of the immune system. If so, a dose effect of gluten would be reasonable. Furthermore, infections drive the immune system towards a Th1-type response, which is also typical for CD. However, the present evidence is not sufficient to allow firm conclusions regarding the underlying mechanisms.

**Breastfeeding Plays a Preventive Role**

The immune defense is not fully developed at birth. In the breastfed infant this is compensated for by immunity transferred from the mother to the infant via the milk [34, 35]. It therefore seems reasonable that the introduction of a dietary antigen while the child is still being breastfed might increase the likelihood of developing oral tolerance to that antigen. However, the role of breastfeeding in the prevention of IgE-mediated allergies is controversial.

Already in the 1950s, based on case series, it was suggested that breastfeeding delays the onset of CD. Increasing breastfeeding rate was also suggested as a possible factor contributing to the declining incidence of CD in the early 1970s in the United Kingdom [19, 20]. However, the study designs used did not enable adjustment of other potentially causal factors.

In the 1980s, Italian case-referent studies [21, 22] revealed that CD cases were breastfed for a shorter duration than referents. This was confirmed in Swedish [15] and German [36] studies. However, a shortcoming of these studies is that they did not clarify whether breastfeeding had a direct causal effect, or if the protective effect was indirect resulting from the postponed introduction of infant formula (i.e. cow’s milk protein), or if it resulted from a reduction in the amount of dietary gluten ingested at an early age, i.e. a dose effect.

In our incident case-referent study the above-mentioned constraints were eliminated [11]. The main finding was that the risk of CD was reduced if the child was being breastfed during the time period when gluten-containing foods were introduced (OR = 0.59, 95% CI 0.42–0.83). This protective effect was even more pronounced if the child continued to be breastfed beyond the period of gluten introduction (OR = 0.36, 95% CI 0.26–0.51), with an increasing effect for every month of breastfeeding. These risk estimates are adjusted for the age of the infant when gluten was introduced into the diet.
and the amount of gluten given. Moreover, a protective effect of breastfeeding was also supported by our ecological study using aggregated data to explore any temporal relationship between the changes over time in incidence rate and changes in infant dietary patterns [7]. Both the rise and later fall in the incidence of CD were temporally related to a change in the proportion of infants introduced to gluten while still being breastfed.

It is important to note that at the time of these studies the majority of Swedish infants were being breastfed for 6 months or longer, i.e. most of the infants were introduced to cow's milk products and other foods while still being breastfed. Also, for most infants the termination of breastfeeding did not coincide with the introduction of infant formula, but rather with increased ingestion of complementary foods. Thus, these findings strongly support breastfeeding as directly reducing the risk of CD, and not merely influencing the risk indirectly through changes in other exposures (fig. 2).

Conclusions

Breastfeeding during the dietary introduction of gluten is protective against CD, as supported by several epidemiological studies of different design. Moreover, this protective effect is biologically plausible taking into account our present knowledge of breast milk composition and the impact of breastfeeding on immune responses, along with current knowledge concerning the pathogenesis of CD. A gradual introduction of gluten also seems to be beneficial. The exact mechanism behind the protective effect of breastfeeding is not yet known. However, it is tempting to speculate that this could be mediated either by reducing the number of infections or by the immune-modulating effect of breast milk, for instance by providing downregulatory transforming growth factor-β1. Our observation that CD patients often have rod-shaped bacteria adhering to the mucosa might suggest that the effect could be mediated via an effect on the bacterial colonization of the gut.

References

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Discussion

Dr. Branski: I have two remarks to make. One, I don’t think that you are speaking about prevention of celiac disease, it is mainly postponing or delaying its presentation, and this is according to the studies from Tampere, Finland. The presentation of celiac disease in Finland was much later mainly with extraintestinal manifestations due to secondary phenomena like diabetes, arthritis, etc. [1]. The other remark I would like to make is that environmental factors are very important for the timing of the presentation and some of these environmental factors are infectious agents like viruses, not necessarily bacteria. I would like to remind the audience of the paper by Kagnoff et al. [2] many years ago about the enteric type of adenovirus; it was supposed to be one of the causes affecting the timing of presentation of celiac disease.

Dr. Hernell: Of course you may be correct. That was what I tried to stress. We really don't know if there has been a true change in the incidence, or if after 1997 we have more cases with silent disease or more cases which contract the disease after 2 years of age. That is why the screening study I was mentioning is so important. However, I don’t think that you can conclude yet that there is no change in the incidence. Although, there is evidence to suggest from the screening studies that have been done around the world that 1% of the population have celiac disease, few studies have been large enough with confidence intervals allowing a firm conclusion of the true prevalence of the disease in different populations. So I think we don’t really know. It may be that you are correct but it could also be that we actually have a chance for primary prevention depending on how we introduce gluten. And your second question?

Dr. Branski: It was a comment regarding the infectious etiology for the timing of presentation.

Dr. Hernell: This observation by Kagnoff with adenovirus 12 has never really been proven or refuted. We have now found that there is a strong association between repeated infections and increased disease risk. I don’t think this is surprising. It could be that infections cause a decreased barrier function with increased penetration of antigens, and if that is combined with an increased antigen load, then it is not unreasonable that an enhanced risk for disease would be found. It could also be that infections shift the immune response from a Th2-type to a Th1-type response. That could also contribute to the development of celiac disease. However, it would be interesting to know whether there are certain infections that are more important than others.

Dr. Sinaasappel: Thank you very much for this provocative presentation. The question I have regards the chicken over the egg question for this bacteria, which is also the case for the cytokines. The last point, and for me you are a little bit provocative, regards starting tolerance induction by introducing gluten during breastfeeding. Is that not what was done during your epidemic?
Dr. Hernell: Your first question was whether the cytokine profile is a secondary phenomenon. I don’t think so. Our studies are based on three biopsies (the old ESPGHAN criteria) and we find the same principle cytokine pattern in patients with treated disease (gluten-free diet), which is clearly different from the pattern in controls.

Dr. Sinaasappel: The kind of bacteria, what types of bacteria did you find?

Dr. Hernell: We are working on that. We are screening all bacteria with 16S rRNA. At this point we believe that it is not just any bacteria. It seems that some bacteria are more common than others. Whether there are just a few bacteria that are adhering is too early to say.

Dr. Sinaasappel: My main point is the introduction of gluten during breastfeeding. You have probably seen a number of cases during your epidemic in whom gluten was introduced at that time because the number being breastfed was decreasing.

Dr. Hernell: I think the beauty of the study is that it is a case-control study and we had the chance to control for confounders. Doing so the strongest preventive factor is to introduce gluten during breastfeeding. We could even see that when controlling for the amount of gluten, every month of breastfeeding after the first introduction of gluten added to the preventive effect. The problem is that too few mothers breastfeed up to 8–9 months to allow sufficient statistical power to the analyses, but there is a clear tendency that each month after the introduction adds to the preventive effect.

Dr. Paerregaard: You certainly convinced us that it is recommendable to proceed with breastfeeding after the introduction of gluten to the diet, but the question of timing and the amount of gluten remains to be settled. You recommend that small to medium amounts of gluten be introduced instead of large amounts. When I look at the comparative studies that have been performed between infants in southern Sweden and Denmark, it was certainly found that the amount of gluten was of importance. However, it was also demonstrated that the quantities the Swedes considered to be small to medium amounts, the Danes considered to be huge. Have you any data to recommend specific amounts of gluten with regard to formula and complementary feeding?

Dr. Hernell: No we don’t have that. The only data we have are from this case-referent study and the way that we chose to define large as compared to small and medium was as I told you. We used the distribution of intake among the controls and set an arbitrary upper limit at their upper one third of intake, which was defined as the large amount. I can’t give you an exact figure of what would be the ideal amount of gluten for the introduction.

Dr. Taminiau: You didn’t mention the viruses. Dr. Heyman has shown that rotavirus enhances the endocytotic uptake of proteins during rotavirus infection, and also shown that during the active phase of celiac disease whole proteins are taken up by endocytosis, much more than in the treated phase. I think what you showed is very interesting, but I still think the viruses might be important and the timing of the rotavirus infection, the protection of the breastfeeding, etc.

Dr. Hernell: I completely agree. What I showed was that the number of infections matters, and our first thought was that these infections were gastrointestinal, in most cases rotavirus. However, it turned out that it was any kind of infection that mattered. Infections probably affect the mucous membranes so it could be rotavirus, it could be other viruses.

Dr. Taminiau: Because the rotavirus also influences the blood cells during the infection.

Dr. Hernell: With rotavirus infections there is a fairly long period after the acute infection of increased uptake of large protein molecules.

Dr. H. Hoekstra: I have a question regarding infections. Often clinicians make the diagnosis of celiac disease in a situation with continuous diarrhea following an acute
infection. Clinical awareness at this time will be very high. My question is: at what time of year, in what month, was the diagnosis of celiac disease made? Could it be related to more clinical awareness?

Dr. Hernell: This is a good question. Because we were aware of that we chose to use the number of infections during the first 6 months, which was before introduction of gluten. Thus, the cases were diagnosed long after the infections that were used in the calculation.

Dr. Vaarala: I found your theory about infections very interesting. There are some studies showing that the MHC genotype has an effect on intestinal colonization, at least in animal models. My question is whether you found any relation between this occurrence of rod-shaped bacteria and the HLA genotype in your patients?

Dr. Hernell: We haven't looked at that; but it is a very good suggestion.

Dr. Benninga: You showed us that the gluten-free diet changed something in the interferon-γ (IFN-γ) cells, etc. We also looked at our treated celiac disease patients, but still found a rise in intraepithelial cells. The question is, how are you sure that all these people did take a gluten-free diet?

Dr. Hernell: The only answer to that is that we had antibody responses and as I showed you those who had their first biopsy had elevated levels of both anti-endomysium and anti-gliadin antibodies and those who had their second biopsy after examination had normal antibody levels and normal mucosal morphology. But of course you can never be 100% sure that they really adhere to the diet suggested, that is correct.

Dr. Schmitz: You gave your advice in the context of the Scandinavian way of feeding infants. What would you advise in countries with a much shorter breastfeeding duration, for example if breastfeeding stops at around 3 months of age, would you say that gluten should be given at 2 months of age or at 6 months of age or later? Is it possible to give general advice?

Dr. Hernell: I have heard this question several times but not exactly as you put it. I am afraid that I do not have an answer. However, the common question is whether or not there is a conflict between our national recommendation to introduce gluten between 4 and 6 months of age and the WHO recommendation of exclusive breastfeeding for 6 months. I don't think that anyone has a final answer. If one follows the WHO recommendation it should not be a problem to postpone the introduction to 6 months as long as the mother continues to breastfeed beyond 6 months. We didn't find that there was a specific age of introduction at which the risk was increased. Others have discussed whether there is an immunological window during which the chance to develop oral tolerance is greater than before or after. I think there are no convincing data in humans. Based on the evidence that we have, and I am speaking only for celiac disease, it seems that if you want to reduce the risk you should introduce gluten while the mother is still breastfeeding, and if she chooses or is forced to stop breastfeeding before 6 months you should really consider whether to advise her to introduce gluten before she stops breastfeeding.

Dr. M. Hoekstra: I think it is very difficult to give a clear advice on this although many people try to ask for this. Is one of the reasons for this difficulty that your data are derived from observational studies and not from intervention studies? So it is not yet exactly possible to indicate what choices should be made here.

Dr. Hernell: That is correct. I think however that it would be very difficult to do a proper intervention study although that would be the ideal situation. You would need to consider genetics and the amount of gluten during the introduction and many other things. It would be a very difficult study to carry out.
**Dr. Bueno:** You mentioned that IFN-γ plays an important role after previous infection. Since IFN-γ increases gut paracellular permeability and subsequently may affect the uptake of gluten, it appears of paramount importance to take into account previous infectious experience or background of infection when introducing gluten to food.

**Dr. Hernell:** Most people do not become intolerant to gluten, so something is obviously wrong with those who develop celiac disease. I think that is a normal function for IFN-γ to actually survey the epithelial surface against the food antigens. But it is correct, if there is increased IFN-γ production there will be a leaky epithelium. That is probably one part of this in which an escalation occurs with more antigens penetrating, and then possibly the problem is that there is no upregulation of TGF-β1 to balance the IFN-γ increase.

**Dr. Keller:** I have a question on the differences in clinical presentation of celiac disease patients. All of us have met with less classical cases and more so-called atypical cases like osteoporosis and so on. Is the availability of celiac antibody serology the only explanation for that, and do you think there is a difference between these atypical presentations in comparison to the classical ones?

**Dr. Hernell:** I don’t know. If we go back and look at what happened for instance when the recommendations were changed in the UK, I think it was at the middle 1970s, it seemed as if celiac disease was actually disappearing. The same change in recommendations occurred simultaneously in Sweden and there was no change in the number of cases diagnosed. So I think there are many factors contributing to the change in the expression of the disease; it is not a single factor.

**Dr. Taminiau:** On the genetic manipulation prevalence, it was shown that in barley, in wheat and also in oats, there are so many epitopes stimulating T cells that it will be almost impossible to manipulate the proteins in the future to get rid of celiac disease.

**Dr. Hernell:** That was something that I put on the slide. But I think that it will be a long time before we have a chance to actually eliminate all the epitopes. It also seems possible that not all patients are reacting to the same epitopes.

**Dr. M. Hoekstra:** What I still do not understand is that if you look at the revised hygiene hypothesis then this hypothesis teaches us that we should no longer speak about Th1- or Th2-mediated disease but in general of a group of immune-mediated diseases, and the chance in a lifetime that you develop such a disease depends on whether you succeeded in developing enough regulatory T cells in early life. But what puzzles me is that if this is correct and if IL-10 is one of the most important indicators of regulatory T cells, that IL-10 decreases if you treat celiac disease. I would expect exactly the opposite. I mean if you treat the inflammation, if that is mediated by regulatory T cells, then you would expect an increase in IL-10-producing cells in biopsies, but you see just the opposite.

**Dr. Hernell:** Actually in active disease there is an increase in IL-10 but not of TGF-β, and there are probably different populations of regulatory cells that express IL-10 and TGF-β. So I think it is more interesting why the regulatory cells expressing TGF-β are not increased, or are they increased but not functioning the way we would expect? I don’t know, I am not an immunologist.

**Dr. M. Hoekstra:** I would like to thank Dr. Hernell for his challenging presentation. We have heard a lot about the developing immune system and about intervention during infancy and the period of weaning. But in my opinion it is not yet possible to draw firm conclusions on the timing of solids and on the administration of probiotics and which probiotics, but we will hear more about that in one of the next sessions. From the last presentation, the relationship between the introduction of gluten during or after breastfeeding has not yet been elucidated.
References
