Early Feeding Practices and their Impact on Development of Celiac Disease

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Abstract

Celiac disease (CD) is an immune-mediated enteropathy triggered by the ingestion of gluten in genetically susceptible individuals. Gluten is a protein component in wheat and other cereals like rye and barley that are generally introduced in the infant's diet at weaning. At present, two schools of thought claim that changing early feeding regimens in at-risk infants can either prevent the onset of the disease or merely delete its onset. Recent advances have increased our understanding of the molecular basis of this disorder and provide the rationale to perform prospective dietary interventional studies to establish the proper timing of gluten exposure to minimize the risk of developing CD

Introduction

CD, or gluten sensitive enteropathy, is an immune mediated chronic enteropathy with a wide range of presenting manifestations of variable severity. It is triggered by the ingestion of gliadin fraction of wheat gluten and similar alcohol-soluble proteins (prolamines) of barley and rye in genetically susceptible subjects with subsequent immune reaction leading to small bowel inflammation and normalization of the villous architecture in response to a gluten-free diet (GFD). CD not only affects the gut, but it is a systemic disease that may cause injury to the skin, liver, joints, brain, heart, and other organs. It is a complex genetic disorder and HLA status appears to be the strongest genetic determinant of risk for celiac autoimmunity. There is a propensity for individuals with CD to carry specific HLA class II alleles, which has been estimated to account for up to 40% of the genetic load [1]. In affected individuals, 95% have either DQ2 (*HLA-DQA1*05-DQB1*02*) or DQ8 (*HLA-DQA1*03-DQB1*0302*), in comparison with the general population in which 39.5% have either DQ2 or DQ8 [2].

CD is now considered to be a T-cell–mediated, chronic inflammatory disorder with an autoimmune component. Altered processing by intraluminal enzymes, changes in intestinal permeability, and activation of innate immunity mechanisms seems to precede the activation of the adaptive immune response [3]. It is the interplay between genes (both HLA and non-HLA associated) and environment (i.e. gluten) that leads to the intestinal damage typical of the disease [4]. Under physiological circumstances this interplay is prevented by competent intercellular tight junctions (tj), structures that limit the passage of macromolecules (including gluten) across the intestinal epithelial barrier. Recent evidence suggest that the gluten-induced up-regulation of zonulin, a recently

described intestinal peptide involved in tj regulation, is responsible, at least in part, for the aberrant increase in gut permeability characteristic of the early phase of CD [4] and the subsequent abnormal passage of gluten into the lamina propria. Here, the protein is deamidated by tissue transglutaminase and is then recognized by HLA-DQ2/DQ8 bearing antigen presenting cells, thereby triggering the onset of the CD autoimmune reaction [3].

Epidemiology

The epidemiology of CD has been entirely rewritten during these last decades [5-8]. In the past, CD was considered a rare disorder, mostly affecting individuals of European origin, usually characterized by onset during the first years of life. By the way, this paradigm is still widely diffused, so much so that in many European countries CD is included in the list of rare disorders protected by specific regulations of the health care system. On the other hand, a large number of studies have recently shown that CD is one of the commonest, lifelong disorders affecting mankind all over the world (with some remarkable exceptions). Currently most cases remain undiagnosed, due to lack of typical symptoms, and can be recognized only through serological screening by sensitive tools, e.g. serum anti-transglutaminase (tTG) determination. CD is not only frequent in developed countries, but is increasingly found in areas of the developing world, such as North Africa and India. CD can contribute substantially to childhood morbidity and mortality in many developing countries.

Nowadays the role of epidemiology has gone well beyond the mere measurement of CD occurrence. A number of situations have been found to predispose to CD, e.g. family history of disease, associated autoimmune disorders or Down syndrome. The

characterization of these at-risk factors is not only important for diagnostic purposes, but also sheds light upon CD pathophysiology and prevention. Furthermore, epidemiological studies have highlighted the role of infant nutrition, particularly age of introduction of cereals and amount of ingested gluten, on the predisposition to CD development. This knowledge could bear implications for human nutrition at large.

Natural History of Celiac Disease

Recent reports suggested that the prevalence of CD in Western countries has been increasing during the past few decades. Rubio-Tapia *et al* found in adult American men a CD serology positive prevalence of 0.2 % in a cohort enrolled between 1948 and 1954 and ~0.9-1 % in a different cohort enrolled recently [9]. The same trend has been noted in Finland, where the overall prevalence of CD in two different population-based samples increased from 1.05 % in 1978-80 to 1.99 % in 2000-01 [10]. We have recently reported the true natural history of CD autoimmunity in the U.S. Our data demonstrated that within an American adult population CD prevalence doubled between 1974 (1 every 501 subjects) and 1989 (1 every 221 subjects) [11]. This trend was further validated by our epidemiological results in a different adult sample screened in 2001, in which we detected a CD prevalence of 1:105, suggesting that during the past 27 years the prevalence of CD among adults in the US increased by 5-fold, doubling approximately every 15 years [11].

Before this study, the natural history of gluten sensitisation in subjects belonging to the general population was unclear. An age-related increase in the prevalence of celiac autoimmunity had only been observed in at-risk individuals, e.g. subjects with a

family history of CD or type 1 diabetes [12-15]. It has been speculated that loss of gluten tolerance leading to immunological and mucosal changes typical of CD usually develops early in life, soon after the exposure to the environmental trigger (i.e. at weaning), while the onset of clinical manifestations of the disease can manifest much later [5]. Conversely, our study demonstrated that loss of gluten tolerance may occur at any time in life for reasons that are currently unclear.

A steady rise in the incidence of autoimmune diseases as well as allergic diseases has been registered in industrialized countries over the last few decades. Both in Europe and the U.S., type 1 diabetes showed a stable and relatively low incidence over the first half of the 20th century, followed by a sharp increase that began some time after the middle of the century [16]. According to the hygiene hypothesis, an early childhood infection or the establishment of mixed intestinal microbiota could down-regulate immunity and suppress different autoimmune disorders [17]. However the raising prevalence of adulthood onset of CD that we observed in our study can be hardly explained by hygienic changes occurring in childhood. Our prospective cohort study in which the same subjects were followed over time also excluded changes in the genetic component as the cause of increased prevalence of CD that we observed since 1974. Therefore, our data provide the undisputable proof that subjects genetically predisposed to CD can loose tolerance to gluten at any age. The amount and the quality of ingested gluten, type and duration of wheat dough fermentation, the spectrum of intestinal microbiota and its changes over time, enteric infections, and stressors in general are all possible switches of the tolerance/immune response balance [8, 18-19]. However further studies are required to clarify the relevance of these factors in causing loss of gluten tolerance and possible

intervention on these factors to prevent the onset of CD and, possibly, other autoimmune diseases in subjects genetically predisposed.

Role of Early Feeding Practice in the Onset of Celiac Disease

Epidemiological data support the hypothesis that early feeding practices may influence the risk of CD development. In Sweden an epidemic of early-onset, typical cases of CD, was observed during the period 1984-1996. The incidence rate of symptomatic CD in children younger than 2 years of age increased 4-fold within a few years and declined in an equally abrupt manner about one decade later. The epidemic was partly explained by changes in infant feeding [20-21]. Factors possibly influencing the disease risk were (1) duration of breast feeding, (2) age at gluten introduction, and (3) type and amount of gluten introduced during the second semester of life.

The effect of breast feeding on CD risk has been recently reviewed by meta-analysis of available studies. It was found that children being breast fed at the time of gluten introduction had a 52 % reduction in risk of developing CD compared with their peers who were not breast fed at the time of gluten introduction. It is biologically likely that the presence of breast milk at the time gluten is introduced increases the chance of developing oral tolerance for the major gluten antigens. An association between increasing duration of breast feeding and reduced risk of CD was also documented. It remains unclear whether breast feeding provides a permanent protection against CD or whether the practice only delays the onset of symptoms [22]. The mechanisms through which breast milk protects against the development of CD could include: (a) reduction of gluten intake, (b) prevention of gastrointestinal infections, and (c) protection conferred by

human milk factors, e.g. secretory IgA, stimulating maturation of the intestinal barrier and down-regulation of inflammatory immune responses.

The relationship between timing of gluten introduction and CD risk is still controversial. This issue was recently investigated in a prospective, observational study conducted in Denver, Colorado (USA), on 1560 children at increased risk for CD or type 1 diabetes. Children exposed to gluten-containing cereals in the first 3 months of life had a 5-fold increased risk of celiac serum autoimmunity (CDA) compared with children exposed to gluten-containing foods at 4 to 6 months. Children not exposed to gluten until the seventh month or later had a marginally increased risk of CDA compared with those exposed at 4 to 6 months (HR, 1.87; 95% CI, 0.97-3.60). Based on these results authors suggested that a favorable "window of exposure" to gluten exists between 4 to 6 months. Outside of this period gluten introduction may increase CD autoimmunity risk in susceptible children [23]. The "tolerance window" hypothesis has been incorporated in the recent recommendations on complementary feeding formulated by the ESPGHAN Committee on nutrition. According to this group of experts, it is prudent to avoid both early (<4 months) and late (>=7 months) introduction of gluten and to introduce gluten gradually while the infant is still breast-fed because this may reduce the risk of CD, type 1 diabetes mellitus, and wheat allergy [24]. .However the "gluten tolerance window" hypothesis has not found confirmation in a similar prospective study performed on 1,511 genetically at-risk German infants. Neither the breast-feeding pattern nor the introduction of formula milk, gluten-containing or gluten-free solid food supplements during the first 3 months of life were associated with an increased risk of CD serum antibodies in this German study [25]. Antigen avoiding is a widely used tool for primary prevention of allergic disorders

in children [26]. The human intestine shows a postnatal developmental pattern of the intestinal barrier function that resembles gut closure observed in other mammals [27]. However, the possibility that delayed gluten introduction may reduce the risk of CD development has never been prospectively investigated.

Finally, the previously mentioned epidemic of CD among Swedish children observed in the mid 80s suggested also that the amount of gluten ingested during weaning can play a pivotal role in the development of CD. The regional differences in the epidemiology of CD in India also give support to the hypothesis that the amount of gluten plays an important role in the onset of CD. CD is reported frequently in high wheat-consuming states in Northern India and quite rarely in the Southern States, where rice is the staple food [28].

Intervention on the infant dietary pattern to change the risk of CD

As previously summarized, several retrospective studies have suggested that the time of gluten introduction in the diet of infants at risk for CD may affect the incidence of the disease. However, the data supporting this hypothesis are circumstantial, limited by their retrospective design, and often criticized by alternative interpretations suggesting that the delay in gluten exposure merely postpones the onset of symptoms rather than preventing the disease. Due to the cross-sectional design of these studies, it remains unclear whether the reported microbial associations (see below) are pathogenic or merely the consequence of CD intestinal inflammation. In order to clarify the role of infant nutrition on the risk of CD development, at least two prospective, intervention studies have been recently initiated. The results of these long-term studies will be available in the next years.

The family study of PREVENTCD: This study is currently performed in 10 European countries and a total of 1,000 children will be involved. The participating children and mothers will be followed for a period of 1-3 years. The project will study the influence of the dietary history in the prevention of CD. The general concept is that small amounts of food substances are administered gradually to "teach" the immune system not to respond to this foodstuff. This is also called "desensitization' or 'induction of tolerance". Newborns from family at risk of CD that are exclusively breast fed and HLA-DQ2 or DQ8 positive are given 100 mg of gluten between 4 and 6 months of age. After 6 months of age, gluten is gradually introduced into their diet. CD autoantibodies are then monitored every 3-6 months to disclose gluten sensitization.

The Italian baby study. This is another initiative aimed at evaluating the role of (a) age at gluten introduction on CD-related autoimmune serological changes in a large cohort of at-risk infants (first-degree relatives of patients with CD); (b) other early environmental factors, particularly milk feeding; (c) different HLA-DQ2/DQ8 genotypes (high risk versus low risk) on CD predisposition, and their interplay with infant nutrition patterns. Between October 2004 and June 2007, 722 infants (51% M) at increased risk for CD were enrolled in this prospective, multicenter intervention study conducted in Italy. At weaning infants were blindly assigned to introduce gluten in their diet either between the 4th and 6th month (group A) or after the 12th month (group B), then entered a follow-up period of 5 years. Diet (duration of breastfeeding and types of formulas, adherence to the dietary plan, amount of gluten ingested) and clinical data were collected during telephone

or face-to-face interviews at 4, 7, 9, 12 months of age. CD serology (IgA anti-transglutaminase antibodies) was tested at 15 (plus HLA-DQ genotype), 24, 36 and 60 months of age. Small intestinal biopsy was recommended in all infants showing positivity of CD serological tests (Fig. 1). At the last study update (October 2008) duration of follow-up was at least 15 m in 100%, 15-24 m in 93%, 24-36 m in 81% and longer than 36 m in 48%. Fifty two percent infants were enrolled in group A and 48% in group B. Prevalence of biopsy-proven CD at 36 m was 8% in group A and 2% in group B ($p<0.01$). At 3 years of age the proportion of infants developing biopsy-proven CD was significantly higher among those weaned with gluten at 6 than at 12 months of age. A longer follow-up is required to clarify whether the delayed gluten introduction effectively protects from CD development or merely delays the onset of the disease.

Intestinal Microbiota and Onset of Celiac Disease

One follow-up study of intestinal colonization process of the microbiota was conducted in 20 Swedish children stratified in high, intermediate and low genetic risk of developing CD. Total bacterial proportions were significantly higher in the high and intermediate genetic risk group than in the low genetic risk group. Gram-negative bacteria and Bacteroides-Prevotella proportions were higher in the high genetic risk group than in the intermediate and low genetic risk groups. In this study the analysis of the fecal microbiota was conducted by fluorescence in situ hibridization and flow cytometry [20]. Both phenotypic methods present a substantial amount of variability and may rely on an individual and subjective interpretation, while the 16S rDNA sequencing, based on ribosomal SSU species-specific variability, has become the qualitative reference

technique for bacterial taxonomy and identification [21].

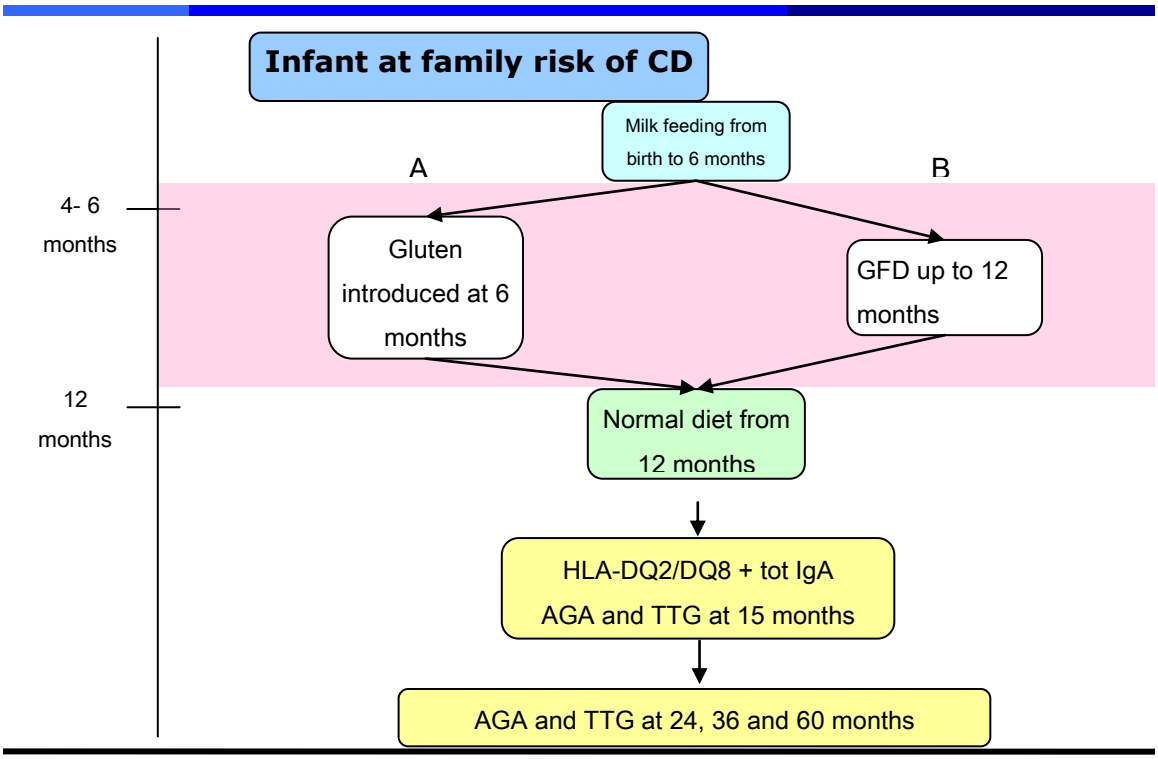
In healthy infants, as described by Palmer et al. [22], Bacteroidetes colonize and establish in the GI tract. Although vary from baby to baby in the timing of their first appearance, they are consistently present in nearly all infants by 24 months. The healthy microbiota evolves during different life stages and in infants shows a lower ratio of Firmicutes to Bacteroidetes than in adults. Overall the microbial ecosystems in each healthy baby achieve stability converging toward a profile characteristic of the adult GI tract in the first year of life [22]. Conversely, our recent prospective studies on the gut microbiome of infants at risk for CD suggest that the microbial ecosystem is different than that of non-predisposed children (Ravel J. and Fasano A, personal communication). Our studies revealed that the colonization process is very dynamic, with high degree of inter-subjects variation over time. Unlike non-predisposed children, the gastrointestinal microbiota of CD at-risk infants does not stabilized towards an adult-like microbiota. Members of the phylum *Bacteroides* are absent from the GI microbiota up to 24 months, while they are predominant in non-predisposed children. These data suggest that early dietary and/or probiotic interventions may potentially stabilize the gut microbiota of these at-risk children, so preventing and/or delaying the onset of CD.

Conclusions

Given the undisputable role of gluten in causing inflammation and immune-mediated tissue damage, CD represents a unique model of autoimmunity in which, in contrast to most other autoimmune diseases, a close genetic association with HLA genes (DQ2 and/or DQ8), a highly specific humoral autoimmune response (autoantibodies to tissue

transglutaminase), and, most importantly, the triggering environmental factor (gluten), are known. This information provides the rationale for the treatment of the disease (gluten free diet) and for preventive interventions based on changes of early feeding practices or changes in gut microbiota. Large, multi-center dietary interventional studies and long follow ups are necessary to generate proper evidence to change current dietary guidelines.

Fig. 1. Study-design of the Italyan baby study. A: group A; B: group B. GFD: gluten-free diet; TTG: IgA anti transglutaminase antibody; AGA: IgG antigliadin antibodies.



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