Early Feeding Practices and Their Impact on Development of Celiac Disease

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Abstract

Celiac disease 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, including 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 delay it. 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 celiac disease.

Introduction

Celiac disease (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. 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 have 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 contrast to 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 seem 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, structures that limit the passage of macromolecules (including gluten) across the intestinal epithelial barrier. Recent evidence suggests that the gluten-induced upregulation of zonulin, a recently described intestinal peptide involved in tight junction 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, to the extent 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 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 at introduction of cereals and amount of ingested gluten, in 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. [9] 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. 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 1978–1980 to 1.99% in 2000–2001 [10]. We have recently reported the true natural history of CD autoimmunity in the US. 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 5-fold, doubling approximately every 15 years [11].

Before this study, the natural history of gluten sensitization 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 US, 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 downregulate 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 lose 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, fur-
ther 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 genetically pre-
disposed subjects.

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 1984–1996 period. 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 man-
ner 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 breastfeeding, (2) age at gluten introduction, and (3) type and
amount of gluten introduced during the second semester of life.

The effect of breastfeeding on CD risk has been recently reviewed by
meta-analysis of available studies. It was found that children being breastfed
at the time of gluten introduction had a 52% reduction in the risk of devel-
oping CD compared with their peers who were not breastfed 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 toler-
ance for the major gluten antigens. An association between increasing dura-
tion of breastfeeding and reduced risk of CD was also documented. It remains
unclear whether breastfeeding provides a permanent protection against CD
or whether the practice only delays the onset of symptoms [22]. The mechan-
isms through which breast milk protects against the development of CD
could include: (a) reduction in gluten intake, (b) prevention of gastrointesti-
nal infections, and (c) protection conferred by human milk factors, e.g. secre-
tory IgA, stimulating maturation of the intestinal barrier and downregulation
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, observa-
tional study conducted in Denver, Colo., USA, on 1,560 children at increased
risk for CD or type 1 diabetes. Children exposed to gluten-containing cere-
als in the first 3 months of life had a 5-fold increased risk of celiac serum autoimmunity compared with children exposed to gluten-containing foods at 4–6 months. Children not exposed to gluten until the 7th month or later had a marginally increased risk of celiac serum autoimmunity compared with those exposed at 4–6 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 and 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 breastfed 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 breastfeeding pattern nor the introduction of formula milk and gluten-containing or gluten-free solid food supplements during the first 3 months of life was associated with an increased risk of CD serum antibodies in this German study [25]. Antigen avoidance 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-1980s also suggested 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 in the Infant Dietary Pattern to Change the Risk of Celiac Disease**

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 recently been 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 on 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 breastfed 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 current status of PREVENTCD is that recruitment of the 1,000 infants has been concluded and now longitudinal observation and analysis are ongoing.

The Italian Baby Study
This is another initiative aimed at evaluating the role of (a) age at gluten introduction in 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 vs. low risk) in CD predisposition, and their interplay with infant nutrition patterns.

Between October 2004 and June 2007, 722 infants (51% male) at increased risk for CD were enrolled in this prospective, multicenter intervention study conducted in Italy. At weaning, gluten was introduced in a blind manner in the infants’ diet either between the 4th and 6th month (group A) or after the 12th month (group B), then the infants were followed up for 5 years. Diet (duration of breastfeeding and types of formulae, 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 and 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 that had positive CD serological tests (fig. 1). At the last study update (October 2008), duration of follow-up was at least 15 months in 100%, 15–24 months in 93%, 24–36 months in 81% and longer than 36 months in 48%. Fifty-two percent of infants were enrolled in group A and 48% in group B. Prevalence of biopsy-proven CD at 36 months 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 according to the 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 hybridization 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. [29], *Bacteroides* colonize and establish in the GI tract. Although the timing of their first appearance varies from baby to baby, 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 *Bacteroides* 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 their microbial ecosystem is different than that of nonpredisposed children [Ravel and Fasano, pers. commun.]. Our studies revealed that the colonization process is very dynamic, with high degree of intersubject variation over time. Unlike in nonpredisposed children, the gastrointestinal microbiota of infants at risk for CD does not stabilize towards an adult-like microbiota. Members of the phylum *Bacteroides* are absent from the GI microbiota for up to 24 months, while they are predominant in nonpredisposed children. These data suggest that early dietary and/or probiotic interventions may potentially stabilize the gut microbiota of these at-risk children, and so prevent and/or delay 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 in early feeding practices or changes in gut microbiota. Large, multicenter dietary Interventional studies and long follow-ups are necessary to generate proper evidence to change current dietary guidelines.

**References**

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Discussion

Dr. Szajewska: You kindly mentioned the PREVENTCD study funded by the EU. I just want to clarify that it’s not an open study; it’s a double-blind placebo-controlled study. As in your study, children are screened for HLA DQ2/DQ8, and only those children are included in our project. The recruitment phase is now finalized. We recruited more than 1,000 infants, but of course we are still waiting for the results.

Dr. Fasano: I appreciate that; I took the information from www.clinicaltrials.gov, so you may want to make some clarification there. I didn’t know that PREVENTCD was a double-blind study. Thank you for the clarification.

Dr. Saavedra: Obviously, the intestine is by far the largest microbiome we have. But we also have skin flora or microbiota, and we have respiratory microbiota. We have these epithelial surfaces that are also in contact with the environment. As we heard in the concerns regarding dermal exposure or respiratory exposure, do you think that the mucosal lymphoid system both in the gut and respiratory tract have the same role, have different roles, do they depend on the allergens, do they work the same?

Dr. Fasano: You are right by saying that there are several interfaces with the environment, each having its complex microbiome. Again, just compare the lung with the gut. The lung has a uniform response, whatever is there needs to go away, nothing is supposed to be there. The gut doesn’t have that luxury, it can’t do that. The gut immune system has this unbelievable discriminating capability to decide what stays and what goes, not only just nutrients and microorganisms but also within microorganisms the gut immune system and pattern recognition receptors are able to distinguish between friends (commensals) and foes (potential pathogens). Nevertheless, I totally agree with you that skin and lung microbiota may play a tremendous role in antigen trafficking and, therefore, in the balance between tolerance and immune response.

Dr. Saavedra: My other concern has to do with the nice drawing that you showed at the beginning which shows one clean side of the tube and the other not so clean side of the tube. Unfortunately, all we’re studying is the end of the tube. We know we have microbiota in the small bowel, and going back to what you said about what has changed to the environment, we only started pasteurizing food a hundred years ago. We today eat some fermented food, and we do a small bowel biopsy, and we get an aspirate of $10^6$ CFU, that’s bacterial overgrowth, that’s abnormal, which probably wasn’t just a hundred years ago. But we are not studying the microbiome with the small bowel, and if you look at, for example, where probiotics work more with rotavirus diarrhea, those are small bowel pathogens, not colonic pathogens. How do we study the largest, longest part of the bowel?

Dr. Fasano: I absolutely approve the questions that have been formulated by the NIH when it put in motion this project on defining the human microbiota in the GI tract. I can go even further by saying contrary to the general wisdom we now know that there is a microbiota in the stomach. We always thought it was hard for microorganisms to survive in such harsh environment. Now we realize that this is not true. What is interesting is the concept that the intestine is not like Las Vegas: what happens in the gut (or one its segments) does not stay in the gut (or in that particular segment). In other words, interactions occurring in the colon affect big time the way staff is handled in the small intestine all the way up to the duodenum. There are several studies showing that an inflammatory process in the colon can affect functions in the small intestine and vice versa. Of course, when you have a rotavirus infection that typically affects the proximal small bowel, your colonic microbiota will change. The key question we asked ourselves when we embarked in the gut microbiota project was the relevance of the gut microbiota in the stools as representative of the colonizing microbiota in the GI tract. One of the concerns was that microorganisms in the
stool could be less efficient colonizers that are more likely eliminated with the stools and, therefore, overrepresented in stool specimens as compared to the composition of microbiota resident on the gut mucosa. This issue was put at rest when microbiota on intestinal biopsies was compared to stool microbiota and they resulted quite similar in their composition. But the most important challenge that in my opinion will also offer unbelievable opportunities is the magnitude of the task that we have on the table. Don’t forget that we had to invest many years and millions of dollars to resolve the human genome made by only 30,000 genes. With the microbiome we are dealing with 100 times more genes. What we are able to do now was unthinkable a year ago; in other words, if you would be so kind to give me your stools, in 3 days and for approximately 70 dollars I will give you back the full composition of your gut microbiota. This was unthinkable until recently. So, I am assuming that something is going to happen in terms of mapping out throughout the GI tract the entire microbiota and how nutrition can impact its composition (nutrigenomics) and, therefore, the interplay between our genes and the genes of bacteria living within our GI tract. That I believe will be the most fascinating frontier.

Dr. Villalpando: I was again intrigued by microbiota because the slides show that kids with celiac disease have more Firmicutes and less protobacteria and Bacteroides which are the most abundant Gram-positive bacteria in the gut. Do you think it would be possible to supplement children with celiac disease with prebiotics or probiotics in an attempt to reinforce Bacteroides or something to deal with antibiotics?

Dr. Fasano: There are two aspects to be clarified here. One is that (and here I am purely speculative) we have witnessed this recent epidemic of autoimmune diseases because of the use and abuse of antibiotics that have tremendously impacted the gut microbiota composition in a negative and sustained (as recently shown by several studies) way. This hypothesis reconciles with the observation that the autoimmune epidemic coincided with the introduction and abuse of antibiotics. I would hate to see this happen again with probiotics. I believe probiotics have a tremendous potential but cannot be used promiscuously for whatever condition they seem to work without customizing the right probiotic for the specific dysbiosis, otherwise we will repeat the same mistake we made with antibiotics. So I think the key elements here, and this is what is called personalized medicine, is to study large numbers of patients, stratify the population because I can’t believe that all the individuals are made equal, find the ones that may eventually benefit from a specific intervention because of a specific dysbiosis, customize the probiotics to give to these individuals and objectively evaluate the outcome. That’s what I think would be the most logical approach.

Dr. Simmer: I want to ask about the preterm infants. We do know about the microbiome and we know that we well and truly have mucked it up with our frequent and prolonged use of antibiotics. Is there an increased incidence of food allergies or even celiac disease in preterm infants?

Dr. Fasano: I can’t answer about celiac disease other than in general terms. Preemies have double disadvantage, the one to be most frequently born from C-section and, therefore, to acquire their microbiota from the environment (not preselected to be compatible with or ‘good for’ his or her own genome) rather than from their mother (that in general has preselected a ‘friendly’ microbiome for her genome and, therefore, for her baby) during vaginal delivery. The second disadvantage is that the totality of preemies are on antibiotic treatment while in NICU, so causing further dysbiosis. It’s well known from the literature that preemies have more chance to develop allergies over time and, therefore, it is tantalizing to hypothesize that this is due to improper microbiota. Dr. Guandalini, do you know anything about preemies and celiac disease?

Dr. Guandalini: No, there is no increased prevalence of preemies among patients with celiac disease.
Dr. Simmer: I actually don’t think there is any increased incidence in food allergy either.

Dr. Fasano: There are definitely reports showing increased incidence of atopic dermatitis or multiple food allergies in preterm infants and other reports that dispute this increased risk. Therefore, the issue is not quite settled and the jury is still out there.

Dr. Lack: About food allergies, if you are talking about type 1 IgE-mediated food allergies, you are protected if you are born prematurely. As for mucosal non-IgE allergies, there is the perception that this may be higher, so I think it operates differently for both sorts of allergies.

Dr. Papadopoulou: Going back to *Bacteroides*, C-section was shown to be associated with a complete depletion of *Bacteroides* in the intestinal lumen. Do you know whether C-section is associated with increased prevalence of celiac disease?

Dr. Fasano: Yes, I just told you that there is a group in Canada that showed this increase of celiac disease among kids born by C-section. Again, I don’t want to go more into the discussion about *Bacteroides* role but *Bacteroides* produce a substance, a polysaccharide that has a tremendous positive role in facilitating Treg maturation, so it would be tantalizing to hypothesize that if you don’t have *Bacteroides* there is a defect in Treg and, therefore, suppression against autoimmune responses. I am aware of several groups studying this phenomenon and await the results of their studies.

Dr. Klish: I hadn’t seen those data on *Bacteroides* before, and I find them very fascinating. *Bacteroides*, if I remember my bacteriology, is one of the major players in bile acid metabolism since it produces deconjugase which alters the structure of bile salts and affects the turnover of bile acid. Have you done bile acid assays on these kids to see if the lack of *Bacteroides* in their stool correlates with any abnormalities in bile acid metabolism?

Dr. Fasano: We are currently looking at this aspect. As you correctly mentioned, the composition of the microorganism ‘village’ in the gut, who stays and goes, really depends on so many factors. Again, food is one of them but also the bile acid metabolism and the short chain fatty acid metabolism decide who is going to stay and who goes. So, I believe that *Bacteroides* are definitely susceptible to that kind of change, and we are very much looking into that because that will be a tremendous finding that will explain so many things that we can’t explain right now.

Dr. Haschke: One comment and one question. When I was working in the US in 1980, celiac disease didn’t exist, and my speculation is that the increasing prevalence in the US has a lot to do with the arrival of Italian GI doctors who provided proper education and had the clinical experience. Could you elaborate a little bit on the so called protective effect of breastfeeding which was shown in the Swedish study? What makes breast milk protective, is it the microbiota which are transferred through breast milk?

Dr. Fasano: Honestly, I think that I can only speculate based on the evidence in the literature. Definitely, the breast milk contains substances that favor some microorganisms and make unfavorable environment for other microorganisms, so it definitely influences the microbiota, no question about that. And again, now we start to see well-designed studies that compare the microbiota of kids on breastfeeding and the ones on formula feeding, and it will be interesting to see what is going to come out of these studies. But I think that it would be reductive to say that it’s just simply the effect on the microbiota that makes the difference. I believe that there is also the passive immune response with immunoglobulins present in the breast milk; I really believe that there are molecules that affect antigen trafficking, protection in terms of trophism factors like growth factors that can help to maintain the intestine barrier protected, and again I believe that the other thing that has been overlooked for many years is the capability of the breast milk to train the gut-associated lymphoid tissue.
that is forming and is in a very dynamic and yet crucial time in the first 6 months of life to manage environmental triggers that reach the lamina propria. So, I think that goes way beyond the microbiota composition.

**Dr. Lima:** We made a study in the human milk, and there were different Lactobacillus in the women who were on antibiotics during C-section and the babies who were born by C-section, and it’s very interesting with regard to Lactobacillus salivarius and Lactobacillus rhamnosus. We are reprogramming our microbiota at this moment, and I think it’s very interesting to continue to study what happens with the reprogramming of our babies in this kind of situation. I think it’s very interesting that we also study the microflora in preterm babies and the mothers of the preterm babies; what happens with these, what is the selection in this kind of patients?

**Dr. Fasano:** I completely agree with your statement. Mother Nature made things the way that they are for a reason, and if we intervene in the process by for example giving antibiotics before a C-section, we definitely affect a very finely regulated process of equilibrium that matured during evolution, no question about that. But I need to make a pledge here, and that probably needs to be stressed more and more. I think that this is the time in which we have an extremely powerful capability to understand the unthinkable, how we interplay with the environment and really put more emphasis on the hygiene hypothesis. However, it would be a travesty if we rush an interventions that are not customized by using pre- and/or probiotics without doing our homework. We will make a disservice, we will definitely pay a price, and with this powerful potential tool that we may have to reprogram and put back in balance – the gut microbiota – can turn against us big time, so I think that people in industry, academia, and legislators need to work in concert to make sure that this is done right. The FDA and the NIH took a major step toward the right direction by requesting specific guidelines on how to use probiotics, what kind of stability they have, their composition and so on and so forth.