Autoimmunity and Diet

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Abstract

Whether diet may influence autoimmunity has been the subject of many unsolved debates. Interestingly, growing evidence indicates a large overlap between the mechanisms controlling tolerance to dietary antigens and autoimmunity. To discuss these links, we will focus on two model human diseases. The first one is IPEX syndrome due to mutations in the X-linked \textit{foxp3} gene. Studies of this disease underscore the role of regulatory FOXP3+ T cells in controlling the reactivity against self antigens and the response to dietary proteins in humans. The second is celiac disease, a complex polygenic disease where exposure to dietary wheat proteins can trigger an autoimmune-like attack of the intestine frequently associated with the onset of extra-digestive autoimmune disorders. In the later disease, recent work shed light on the mechanisms that drive the intestinal inflammatory response to gluten and suggests impairment of immunoregulatory mechanisms that control intestinal tolerance and autoimmunity. Yet the exact role of gluten in the pathogenesis of extra-intestinal autoimmunity has not been elucidated. Interestingly, recent work indicates that dietary factors, including vitamin A and breast milk feeding, can protect against the development of harmful responses to dietary proteins. It is unclear whether this protection can apply to the prevention of autoimmunity.

Introduction

Autoimmunity is defined by the onset of pathogenic reactivity against self antigens leading to tissue destruction and disease. Tolerance to autoantigens is achieved by combining elimination of potentially autoreactive T cells during their differentiation in the thymus (central tolerance) and potent peripheral regulatory mechanisms (peripheral tolerance) which downmodulate activation or eliminate autoreactive lymphocytes. In most individuals, these mechanisms successfully prevent harmful reactions against self antigens. Yet, in some others, due to the combination of genetic traits and exposure to
environmental factors, these protective mechanisms are overcome allowing autodestructive inflammation.

Diet is one major environmental contributor to the stimulation of the host immune system bringing approximately 200 g of foreign proteins to adults every day. Robust mechanisms relying on the digestive barrier and on the potent immune system associated with the intestine can prevent adverse reactions to food antigens. Yet, a lapse in these mechanisms can lead to severe adverse responses resulting in either IgE-mediated allergy or T-cell-mediated reactions which can severely damage the intestinal mucosa and compromise digestive functions, as observed in celiac disease (CD). In CD, mechanisms leading to intestinal damage largely overlap with those responsible for tissue damage against self antigens in T-cell-mediated autoimmune diseases. Furthermore, a high proportion of CD develops extra-digestive autoimmunity. One long-lasting question has therefore been the role of the diet and particularly of food proteins in influencing the onset of immune reactions against self antigens.

A large number of already ancient studies have used diverse animal models of autoimmunity and suggested the beneficial effect of caloric restriction, a diet enriched in unsaturated fatty acids, an elementary diet over a cow's milk or gluten-containing diet, or some oligo elements [1]. Most of these studies have remained descriptive and are difficult to translate to the human situation. In contrast, recent data in two human diseases bring interesting clues on the interplay between diet and the immune system. The first one is the IPEX syndrome which provides insight into the community of mechanisms which control autoimmunity and tolerance to food antigens and point to the key role of the subset of regulatory (CD4+CD25+FOXP3+T cells) [2, 3]. The second is CD which represents a privileged model to analyze the link between reactivity against a food antigen and autoimmunity [4]. In the latter disease, epidemiological data provide strong evidence that early diet habits and notably breast milk feeding may influence the risk of developing CD [5]. How breast milk may protect offspring from developing adverse immune reactions will be briefly discussed based on a very recently described mouse model [6].

**Lessons from IPEX Syndrome**

Immune dysregulation polyendocrinopathy enteropathy X-linked syndrome (IPEX) is caused by mutations of the \textit{FOXP3} gene located in Xp11.23, which encodes a 48-kD nuclear factor requested for the generation and/or function of regulatory CD4+CD25+FOXP3T cells. Mutations result in the very early onset of a severe and often lethal disease characterized by a variable combination of autoimmune disorders, type-1 diabetes (TID), autoimmune enteropathy, nephropathy, thyroiditis and dermatitis [3]. Strikingly, two brothers followed in our institution developed an atypical IPEX syndrome
associating autoimmune enteropathy, dermatitis, very high titers of IgE and dramatic allergic reactions to food allergens notably to peanuts and cow's milk [2]. These children and female transmitters in the family harbored a large deletion of the 5' region of the FOXP3 gene. Consistent with a loss of function mutation, these children had almost no circulating CD4+FOXP3+ T cells and no functional regulatory T (Treg) cells. RT-PCR analysis of intestinal cytokines revealed massive and simultaneous upregulation of IL-2, IL-4 and IFN-γ [2]. This observation provides a striking demonstration that in humans, besides their role in preventing autoimmunity, Treg cells are central for tolerance to food antigens and control both Th1 and Th2 responses.

So-called natural Treg differentiate in the thymus from a subset of CD4+ T cells with a relatively high avidity for self antigens and migrate into the periphery where they prevent autoimmune reactions. Induced Treg differentiate in the periphery from naïve CD4+ T cells during immune responses to exogenous antigens and exert in turn a tight retrocontrol, avoiding excessive or protracted immune reactions against these antigens [7]. Recent studies indicate that the intestine is a privileged site for the differentiation of induced Treg [8]. Thus, a subset of intestinal dendritic cells can promote the differentiation of Treg via the release of two soluble factors, retinoic acid and TGF-β [9]. Retinoic acid is a derivative of vitamin A and enzymes converting vitamin A into retinoic acid are selectively upregulated in intestinal dendritic cells [10]. TGF-β is a potent immunoregulatory cytokine instrumental in the prevention of intestinal inflammation [11] and autoimmunity [12]. The mechanisms which impart intestinal dendritic cells with tolerogenic properties have not been fully elucidated but there is strong evidence that they are conditioned by soluble factors released locally, notably by epithelial cells [13]. Intestinal dendritic cells appear instrumental for the induction of active tolerance to the microbiota and to dietary antigens, and experimental data indicate that mucosal dendritic cells that have captured luminal dietary or bacterial antigens can, either in the Peyer's patches or after migration from the lamina propria into mesenteric lymph nodes, induce differentiation of Treg specific for these antigens [8]. Due to their acquisition of homing receptors, these T cells can home back into the mucosa to maintain homeostasis. It is likely that they can also migrate toward the periphery to maintain systemic tolerance, notably to food antigens.

Altogether these data underscore: (a) the overlapping role of Treg in the control of self-reactivity and responses to food antigens; (b) the unique capacities of intestinal dendritic cells to generate Treg against harmless intraluminal antigens. Interestingly, in experimental models of autoimmunity, it has been shown that oral feeding with an autoantigen led to the generation of Treg cells that can prevent subsequent induction of the autoimmune disease by systemic administration of this antigen [14]. Furthermore, oral feeding with myelin basic protein not only prevents the autoimmune encephalitis induced by the systemic administration of this protein but also very signifi-
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cantly inhibits its induction by a distinct neuronal antigen, suggesting that Treg cells elicited in the gut compartment could migrate into an inflammatory site and prevent an advert reaction by a bystander effect [14]. However, it remains difficult to conclude that the generation of Treg cells against dietary antigens can generally contribute to prevent reactivity against self antigens or conversely that failure to generate Treg cells in response to dietary antigens will lead to autoimmunity.

**Lessons from Celiac Disease**

CD is a chronic inflammatory enteropathy induced by proteins from wheat, barley and rye (collectively called gluten) in genetically predisposed individuals. CD differs stricto sensu from an autoimmune disease since intestinal lesions and digestive symptoms are strictly dependent on exposure to gluten and subside on a gluten-free diet. Yet, CD is associated with antibodies against an autoantigen, tissue transglutaminase II (TG2) [15], and a high incidence (>20%) of autoimmune diseases, the onset of which seems to be prevented by a strict gluten-free diet [16]. CD thereby provides a very interesting model to analyze the possible link between immune reactions against food antigens and autoimmunity.

The complementary studies of Sollid's and Koning's groups, reviewed in [4] and [7] have provided definitive evidence that the keystone in CD pathogenesis in the induction of a gliadin-specific CD4+ T-cell response orchestrated by the main genetic risk factors, the MHC class II HLA-DQ2 molecule present in 90% of the patients or the related HLA-DQ8 molecule observed in most of the remaining cases. In this regard, CD provides a striking example on how the encounter between a particular (but not per se pathological) genetic background and a specific environmental factor can drive pathology. Indeed the distinctive composition of gluten proteins characterized by a high content in proline and glutamine residues underlies their elective recognition by the immune system in HLA-DQ2/8 individuals. First, due to the lack of endoprolylpeptidase activity in gastrointestinal and brush border enzymes, digestion of prolamines is incomplete leaving intact large immunogenic fragments which can cross the mucosa. Second, due to the presence of repeats rich in proline and glutamine, prolamines serve as a privileged substrate to Ttgase, an enzyme constitutively expressed in the intestinal lamina propria and transiently activated upon tissue damage. This enzyme can deamidate neutral glutamine into negatively charged glutamic acid, introducing negative charges in gluten peptides which promote their interactions with positively charged residues in the peptide pocket of HLA-DQ2/8, resulting in stable HLA complexes that can be efficiently recognized by T cells [4, 17]. Gluten-specific CD4+ T cells can promote the production of IgA against gluten. Notably, Ttgase can cross-link gluten, and may thus drive the formation of a neoan-
tigen recognized by the immune system, explaining the induction of IgA not only against gliadin but also against this autoantigen. Finally, one characteristic feature of CD4+ T cells in active CD is their production of large amounts of IFN-γ, able to induce severe mucosal damage [4, 17].

However, while HLA-DQ molecules are instrumental to drive the CD4+ gliadin response, it remains at present unclear why and how the anti-gliadin CD4+ T-cell response switches in a subset of HLA-DQ2/8 patients and at very variable periods of time in life from tolerance to pathogenicity. Analysis of mice transgenic for HLA-DQ8 provides interesting insight. Thus, upon oral administration of gluten the late mice develop a strong response to gluten but no enteropathy [18]. Notably, intestinal T cells stimulated by gluten produce TGF-β and IL-10, two immunoregulatory cytokines but no IFN-γ, indicating that efficient antigenic presentation is not sufficient per se to break local tolerance. Furthermore, HLA-DQ8 mice crossed on the autoimmune NOD background develop IgA antibodies against tTG2 and a blistering skin disease mimicking dermatitis herpetiformis but no enteropathy [19], indicating that antigen presentation is sufficient to drive a potent adaptive response likely driving an antibody-mediated skin disease but insufficient to drive a pathogenic intestinal Th1 response. These observations raise questions on the mechanisms which can switch off tolerance and promote intestinal inflammation to gluten.

Several complementary hypotheses are currently being investigated. Obtained results underscore the similarities between CD and bona fide autoimmune diseases. The first hypothesis relies on the role of additional genetic factors. Thus the HLA region only accounts for 40% of the genetic predisposition. Recent genome-wide scan analyses have identified 8 new regions linked to CD, 7 of which contain immune genes. Notably the strongest association is with the 4q27 region [20]. This region contains the genes encoding IL-2 and IL-21, is also associated with TID and autoimmune thyroiditis and is syntenic to the idd3 locus of NOD mice. Notably, the idd3 variant is associated with a decrease in IL-2 production resulting in a lesser number of Treg (IL-2 is a key survival factor for Treg). In humans, the impact of the associated variant on either IL-2 or IL-21 synthesis is unknown but it has been shown that IL-21, which promotes Th1 responses, is overproduced in the duodenum of patients with active CD [21]. However, it should be pointed out that these new variants account for only 3–4% of the genetic predisposition, suggesting a very weak contribution of each variant which will be difficult to delineate [20].

A second hypothesis relies on the triggering role of virus and notably rotavirus. Thus, the onset of CD in childhood has been associated with serological evidence of repeated rotavirus infection, a situation again reminiscent of TID [22]. Via the stimulation of Toll receptor 3, the role of this virus may be to promote the production of α-interferon. This cytokine is a potent inducer of Th1 responses and is over-expressed in the intestine of active CD patients [23].
Finally the last hypothesis derives from the study of intraepithelial lymphocytes (IELs), a population of lymphocytes massively increased in CD and the origin of a rare but most severe complication of CD, T-cell lymphomas. Our work demonstrating that T lymphomas in CD arise from IEL led us to investigate mechanisms impairing their homeostasis [for review see 4]. Our attention was attracted to IL-15 by studies in mice showing that this cytokine controls IEL homeostasis and promotes the onset of T lymphomas. Consistent with a role of IL-15 in CD, we observed that IL-15 is massively upregulated in active CD, both in epithelial and lamina propria cells. Several studies, including ours, suggest that IL-15 plays a central role in the pathogenesis of the intestinal disease. First, IL-15 can deliver a potent anti-apoptotic signal which drives the abnormal survival and accumulation of IEL. Second, IL-15 can stimulate a cytolytic attack of the epithelium by IEL [for review see 4]. Our more recent data indicate that the role of IL-15 extends beyond the activation of IEL. Thus, we have shown that IL-15 can block the effects of TGF-β, a cytokine which exerts a potent retro-control on intestinal inflammation and autoimmunity [24]. In addition, we have recently observed that IL-15 impairs the regulatory effects of Treg [25].

Thus, to summarize, intestinal damage in CD results from both a strong gliadin-specific CD4+ Th1 T-cell response that develops in the lamina propria and a cytolytic attack of the epithelium by CD8+ T IELs. Although the mechanisms that unleash these pathogenic responses are not fully delineated, the abnormal production of IL-15 has an important contribution by stimulating the effector functions of IELs and impairing local immunoregulation. The mechanisms resulting in intestinal damage in CD are thus largely reminiscent of mechanisms thought to drive tissue lesions in autoimmune diseases. In the case of uncomplicated CD however, intestinal damage can be reversed by the gluten-free diet.

That in the vast majority of the patients intestinal damage remains controlled by the eviction of gluten from the diet raises two questions. The first one concerns the mechanisms driving the extra-digestive autoimmune manifestations in CD. These mechanisms may vary depending on the associated autoimmune disease. Strikingly, in dermatitis herpetiformis, there is no T-cell infiltration of the skin but only deposits of IgA directed against a local tTgase (tTgase-3) and of complement suggesting an antibody-mediated mechanism [26]. This hypothesis is supported by findings in transgenic HLA-DQ8×NOD mice [19] (see above). The mechanism driving the production of antibodies specific against skin tTgase remains elusive but likely depends on exposure to the gluten since this manifestation is cured or markedly improved after gluten eviction. In contrast, in diabetes and thyroiditis, tissue damage is more likely driven by T-cell-mediated mechanisms related to those responsible for intestinal damage. In the latter diseases, symptoms cannot be reversed by a gluten free diet but tissue destruction may be irreversible. What is the origin of the putative autoimmune T cells? Can T cells elicited by gluten in the gut
migrate toward these extra-digestive locations, cross-react with autoantigens and drive damage? Alternatively, inflammation in the gut may allow the in situ appearance of autoreactive T cells which can then migrate to other tissues and induce damage that will be perpetuated once initiated. The observation in a recent French study of a lower incidence of autoimmune complications in CD patients who strictly adhere to the diet than in non-compliant patients may support this hypothesis [16]. Yet, it is also possible that genetic predisposing factors common to CD and autoimmune diseases [27] may simultaneously promote an abnormal immune response to gluten in the gut and autoreactivity in the peripheral tissues.

A second question raised by these findings is the capacity of dietary proteins, notably gluten and cow’s milk proteins, to drive the onset of autoimmune diseases in non-CD patients. Numerous studies have addressed this question and more particularly have tried to assess the risk associated with an early exposure in life to these proteins. Results are conflicting [28]. Two very interesting studies suggest that the risk of developing autoimmune antibodies associated with TID is not directly linked to the early exposure to these dietary proteins but rather to the duration of breast milk feeding [29, 30]. These conclusions are in line with observations in CD made during and after an outbreak of CD (so-called CD epidemics in Sweden). Thus a fourfold increase in CD incidence in 2-year-old children was observed between 1985 and 1987, parallel to an average twofold increase in daily gluten consumption and a delayed introduction after the age of 6 months. The incidence declined after 1995 coinciding with an increased proportion of children still breastfed after 6 months (76 vs. 54%), a reduced consumption of gluten and, after 1996, preferential introduction of gluten between 4 and 6 months preferably during breastfeeding [31].

The protective effect of breastfeeding on the development of tolerance is supported by a very recent study in a mouse model which shows that exposure of lactating mothers to an airborne antigen can prevent the induction of asthma by this antigen in their offspring. The authors showed that OVA is present in the milk of exposed mothers at concentrations comparable to those of dietary proteins in human milk. They elegantly demonstrated that tolerance induced by breastfeeding relied on the presence of TGF-β in milk and was mediated by CD4+ Treg induced in the offspring by a TGF-β-dependent mechanism [6].

In conclusion, data on the IPEX syndrome underscore the overlap between mechanisms that control autoimmunity and the response to food antigens in humans. Data on CD illustrate the striking overlap between T-cell-mediated damage induced in the gut by gluten in predisposed individuals and T-cell-mediated autoimmune disorders. Notably these two disorders can be associated and/or occur in individuals who share predisposing genetic factors with CD. Thus, should observations in CD be taken as evidence that an intestinal immune reaction to a dietary protein can drive peripheral autoimmunity? Or
should we rather suspect that, given the similarities in the regulatory mechanisms controlling autoimmunity and tolerance to food antigens and the similarities in the genetic background of patients, the two types of diseases, loss of tolerance to food and autoantigens, can develop in parallel, either simultaneously or at different time points depending on the combination of genetic and environmental factors in a given patient. These hypotheses remain open and should be addressed in further studies. Finally, it should be stressed that some dietary factors may influence tolerance to food proteins and perhaps the onset of autoimmunity. Vitamin A has emerged as a key regulator of intestinal functions and can efficiently promote tolerance. Breastfeeding also emerges as a potentially protective factor and recent results offer renewed perspectives for prevention strategies aiming to reduce the risk of CD or allergy. A protective role against extra-digestive autoimmune diseases remains to be demonstrated.

Acknowledgments

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References

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Discussion

Dr. Sartor: What percent of people with HLA-DQ2 and 8 actually develop celiac disease?

Dr. Cerf-Bensussan: Approximately 35% of Caucasians are HLA-DQ2 or 8 and probably no more than 2% of these individuals develop celiac disease. Yet Vader et al. [1] nicely demonstrated a dose effect so that people who are homozygous for HLA-DQ2 have a higher risk of developing celiac disease. In addition patients who develop a very
Dr. Sartor: The transgenic mice give you a tremendous opportunity to stress the system with the concept of mucosal permeability. If you give a nonspecific barrier breaker, a hypertonic solution, which certainly has been used in IL-10 knockout mice with colitis, there will clearly be proximal small bowel ulceration as well. So that would be one mechanism.

Dr. Cerf-Bensussan: Trying to break oral tolerance in mice seems a real challenge in our as yet limited experience. We are currently using mice with a TCR specific for ovalbumin as a model to mimic the DQ2-driven CD4 response of celiac patients and try different regiments to induce the enteropathy. We want to test the factor that we suspect to be important in breaking tolerance in celiac disease, so we are crossing TCR-OVA mice with IL-15 transgenic mice.

Dr. Sartor: So you need to separate the immunoregulatory barrier. Another thought I just had might be that the Epstein-Barr virus and related viruses can block IL-10. Actually forget that one because it’s an agonist rather than an antagonist.

Dr. Cerf-Bensussan: I didn’t discuss the problem of the barrier in my talk due to the lack of time. Martine Heyman in my laboratory has been working on this problem in celiac disease. We have provided evidence of an abnormal transport of gliadin peptides in active celiac disease patients [3]. In contrast to many expectations, there was no entrance of peptides via the paracellular but rather via the transcellular pathway. Thus we have shown that in active celiac disease there is an abnormal retrotransport of gliadin-IgA complexes which allows translocation of intact gliadin peptides into the lamina propria. One attractive hypothesis is that gliadin-IgA complexes can provide a danger signal to dendritic cells and thereby promote the loss of tolerance and result in inflammation. We are working on this hypothesis.

Dr. Sartor: You should look at the same genes that we see in Crohn’s disease and osteocolitis that are involved with barrier defects.

Dr. Cerf-Bensussan: You are right, yet it seems to me that genome-wide analyses in celiac disease and Crohn’s disease show marked differences in the nature of the variant genes concerned. In Crohn’s disease identified variants seem more related to barrier or innate immunity, while genes associated with celiac disease seem more related to adaptive immunity.

Dr. Thornton: You mentioned IL-15 and you touched briefly on IL-2 and IL-21, so really that flags up the obvious connection between them that they are all common γ-chain cytokines. Do you know if there is any genetic variance in the α chain that confers this specificity for each of those cytokines that are associated with celiac disease?

Dr. Cerf-Bensussan: The recent genome-wide associations suggest an association with the region which contains the IL-2 and IL-21 genes [4].

Dr. Thornton: Because you touched on the rotavirus TLR3 story, do you know how TLRs regulate either the expression to cytokines or the activity by their receptors?

Dr. Cerf-Bensussan: Activation of the TLR3 can lead to the production of IFN-α. In mice, recent work has suggested that intraperitoneal injection of poly IC can stimulate TLR3 activation in the intestine and lead to an enteropathy that is dependent on IL-15. There is also evidence of an interplay between IL-15 and IFN-α. In contrast, there is no evidence, to my knowledge, that TLR3 can stimulate IL-21 production [5].

Dr. Heine: Just a question regarding the protective effects of breast milk. Do the levels of IgA and TGF-β expression in breast milk influence the protective effects against food allergy?

Dr. Cerf-Bensussan: You mean the relationship between TGF-β and IgA. TGF-β is necessary to induce the switch from IgM to IgA, so there is a clear link between TGF-β
and IgA at least at the level of the B cells. In the study by Verhasselt et al. [6] the protective effect was not related to the presence of antibodies in the milk but required TGF-β.

**Dr. S. Koletzko:** You gave references that rotavirus infections may trigger the immune response or the outbreak of celiac disease. Now we have a live vaccine which is given orally within the first 6 months of life. Is there any evidence or has anybody looked in animal models whether this live vaccine may increase or decrease the risk in predisposed animals, or when applied to humans for celiac disease?

**Dr. Cerf-Bensussan:** That is a very good question: will it be useful to protect children against rotavirus infections and decrease the incidence of celiac disease or, on the contrary, are we going to increase the incidence? Unfortunately we do not have a good animal model to test this risk. It still has to be proven that rotavirus is important. Current studies aiming to analyze the development of celiac disease in children at risk may help to ascertain the role of rotavirus. If confirmed, your question will have to be addressed in depth.

**Dr. Brandtzaeg:** I think celiac disease is a wonderful model in humans to study the importance of barrier function, or whatever variables are related to homeostasis in the gut. A comment on barrier function; I know there is one recent study showing that some celiac disease and inflammatory bowel disease patients share a polymorphism in a barrier gene related to the tight junctions.

**Dr. Cerf-Bensussan:** You are right, there is an association with a region which contains the gene encoding Myo1XB9B [7]. Yet the polymorphism is present in an untranslated region and there is, to the best of my knowledge, no experimental evidence of a role of Myo1XB in the control of intestinal permeability. So I think we have to be careful about the interpretation of this association.

**Dr. Brandtzaeg:** You also know about the model of Thomas et al. [8] on zonulin production by activated epithelial cells and its receptor interaction in the small intestine which can open up tight junctions. I suppose you don’t like that model.

**Dr. Cerf-Bensussan:** No, it’s not a question of not liking the ideas. I think there is a problem with these data and I don’t want to comment on them.

**Dr. Brandtzaeg:** There is one statement you made which I would like to challenge. You said that when you remove gluten from the diet everything is alright. That is not quite true because, a couple of years ago we published data showing that there is still some activation of the epithelial compartment even after a gluten-free diet for at least 2 years [9].

**Dr. Cerf-Bensussan:** I take your point completely. I have been too caricatural when indicating an improvement under a gluten-free diet.

**Dr. Brandtzaeg:** This is very important because it means that there is a trigger of the epithelium, which we don’t know, actually going on even after gluten removal.

**Dr. Cerf-Bensussan:** Yes, many patients on a gluten-free diet have increased numbers of intraepithelial lymphocytes (IELs) for years. In addition, some patients on a gluten-free diet will become resistant to the diet and develop lymphoma from IELs. So clearly not everything goes back to normal when you remove the gluten but at least the intensity of the stimulation is decreased. The subnormal intestinal architecture is able to recover, the symptoms stop and the risk of complications decreases; notably the incidence of lymphoma and autoimmunity is decreased.

**Dr. Brandtzaeg:** Clinically fine, but if you go on for a mechanism, we showed that CCR9 expression is kept low, which means activation of IELs after 2 years. Then I come to my point: Forsberg et al. [10] in the north of Sweden showed bacterial colonization on the epithelium in celiac disease patients. Do you know anything about this?

**Dr. Cerf-Bensussan:** They showed the presence of rod-shaped bacteria visible in the duodenum of celiac disease patients [10]. It is a question common in inflammatory bowel disease. It is the chicken and egg question. It seems to me that bacteria adhere
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more easily to an inflammatory mucosa. In the case of celiac disease, I would suggest that bacterial adherence is a secondary event but may perpetuate inflammation.

**Dr. Renz:** I am very much intrigued by the data on the recognition of T cells and the development of these transglutaminase peptides, particularly the role of deamination. What comes to mind is that deamination is now considered in many autoimmune diseases like rheumatoid arthritis, the deamination of filagrin peptides, proteins, fibrinogen, and so on. Can you elaborate a little bit on this pathway? Do you also consider this an important pathway in autoimmunity, and what is going wrong there?

**Dr. Cerf-Bensussan:** In this regard celiac disease is clearly an interesting model disease. In the case of celiac disease, deamination of peptides derived from foreign proteins (gliadins or glutenins by transglutaminase) improve their presentation to the immune system. In the case of arthritis, other enzymes can modify self proteins and promote their abnormal recognition by the immune system. Yet I do not know of other autoimmune diseases where transglutaminase is involved. Do you have any ideas or suggestions?

**Dr. Renz:** No, I don't have any suggestions, but it just comes to mind that this has also not been discussed in any other diseases such as rheumatoid arthritis.

**Dr. Cerf-Bensussan:** Transformation of foreign epitopes or self epitopes which enable their recognition by the immune system is a common mechanism in autoimmunity. Yet, I do not know how it works for filagrin.

**Dr. Renz:** Apparently this regulation must be an important pathway.

**Dr. Hernell:** A few comments, the first is about rotavirus. It may not be that it is exclusively rotavirus because what we found is that repeated infections, even if you exclude gastrointestinal infections, are a risk factor. More than 3 infections as compared to less than 3 infections during the first 6 months of life, i.e., even before you start to introduce gluten in the infant's diet, increases the risk, and particularly when combined with a larger dose of gluten consumed during the first 2 weeks after the introduction. It may again be a question of barrier function.

**Dr. Cerf-Bensussan:** I take your point entirely, rotavirus is one example and it could be whatever infection, IL-15 as well as IFN-α are induced by a number of viruses and intracellular pathogens. So any virus or pathogen which can induce IL-15 could certainly do the job.

**Dr. Hernell:** The second point I would like to raise is that celiac patients are really not 'normal' when they are treated with a gluten-free diet. For instance, we found that they do have elevated expression of IFN-γ mRNA by their IELs, and also that they have a different glycosylation pattern than controls that remains when they are on a gluten-free diet. You may wonder if that is a primary effect because we also found these rod-shaped bacteria adhering to the mucosa also when they are on a gluten-free diet. Interestingly enough we don't see that as often now as we did during the epidemic, and it should be born in mind that when we tried to explain the epidemic, we could explain no more than 50% of the increase in diagnosed celiac disease by the feeding pattern. Thus 50% remained to be explained. Now when children are diagnosed at an older age we don't see these rod-shaped bacteria adhering to the mucosa as often as during the epidemic, but there are differences in the microbiota between celiac patients, even treated celiac patients and relatives of celiac patients, and controls. Taken together these findings relate a lot to the microbiota and I think we should not forget that when we discuss the pathogenesis of celiac disease.

**Dr. Cerf-Bensussan:** I do agree when you say that patients on a gluten-free diet are not totally normal. I heard people from Finland indicating that their patients can recover a fully normal mucosa and normal counts of γ-δ IELs, which is never the case in our country where we always observe somewhat elevated titers of IFN-γ, too much IL-15, too many IELs in treated patients. Therefore I have often wondered whether
celiac disease patients in Finland adhere more strictly to their diet than in France. Therefore I am interested in hearing whether the findings are the same in Sweden. The more I think about it, it seems to me that these patients have some self-reactivity in the intestine which is maintained over life, and this should be worked out. Concerning the microbiota, whether bacteria participate in the activation of IEL is an interesting idea which is worth of address.

**Dr. Hernell:** I also have a question because celiac disease is very peculiar in terms of reaction to a food antigen and it seems that most of the IFN-γ is actually produced by the IEL CD8 cells and that seems more typical for an intestinal infection than for reactions to other food allergens, and you can also turn it on or switch it off as you can do with an infection. Why is that?

**Dr. Cerf-Bensussan:** This is a very interesting remark. I am glad you stressed the role of the IELs as we had difficulty having them accepted as an important player in celiac disease. How they are stimulated has not been clearly delineated. IL-15 provides an important activation signal but is likely not sufficient alone. Can IELs recognize gliadin peptides as suggested by the Italian group from Naples? Can they recognize antigens derived from bacteria or from self antigens? IL-15 may then promote their activation, resulting in upregulation of activating NK receptors which in turn will further stimulate the recognition of self antigens induced by stress and/or inflammation. Such reactivity may not subside completely after gluten eviction.

**Dr. Vaidya:** With regard to the Swedish study in which gluten was introduced between 4 and 6 months under the cover of breastfeeding and there was a dramatic decline in celiac disease. When the introduction is done at 6 instead of 4 months, would it be of benefit?

**Dr. Cerf-Bensussan:** The outbreak of celiac disease was associated not only with an increase in consumption of gluten, but the indications given to the mothers to delay the introduction of gluten to after 6 months when breastfeeding was stopped. The Swedish pediatricians decided thereafter to recommend the introduction of smaller amounts of gluten and earlier between 4 and 6 months under breastfeeding, as the vast majority of Swedish mothers breastfeed until 6 months. This was associated with a decrease in the incidence [10], which seems to have returned to the usual level.

**Dr. S. Koletzko:** May I just comment because this is exactly the question which hopefully will be answered by a prospective study funded by the European Union where the children are randomized to receive either placebo or small amounts of gluten starting at the age of 5 months. We had a lot of trouble because this is against the WHO regulations and we had to convince our ethics committee that it is at least according to the European feeding recommendations in infancy.

**Dr. Hernell:** It doesn’t need to be a conflict because the important thing seems to be to introduce gluten while the mother is still breastfeeding and every month of breastfeeding after the introduction seems to confer further protection. So it’s not necessarily a question of whether gluten is introduced between 4 and 6 months, it’s perhaps a question of how long the mothers continue to breastfeed after the introduction.

**Dr. Cerf-Bensussan:** That’s what I understand also. It is actually what the data in mice suggest, if mice can be compared to humans which is sometimes not so easy.

**Dr. Du Toit:** Thank you very much for a very interesting talk. A different disease which you haven’t mentioned and where it is certainly teasing to try and link autoimmunity with food allergenicity is chronic urticaria. There is no good evidence certainly in children and adults. In many studies, between 40 and 60% have autoantibodies against the IgE receptor or IgE itself, and many studies have looked at the role of food in even initiating this process or exacerbating the process, but none of them are convincing. Given the close link between these symptoms and the symptoms of immediate onset food allergy, I think everyone is always tempted to put these patients on
extreme diets. In your experience, are you aware of food playing a role in this condition, either initiating or exacerbating it?

*Dr. Cerf-Bensussan:* I have no experience with this condition, but I am very glad that you mentioned it because it is something that I don’t know.

**References**