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Gliadin as a stimulator of innate responses in celiac disease

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Abstract

In celiac disease (CD) we have the prototype of an immune mediated response dominated by the activation of the adaptive immune system and in particular of CD4+ HLA class II restricted T cells. Various seminal studies have established the precise mechanism of how antigen (prolamine) specific activation of CD4+ mucosal T cells occurs. Thus, CD is a condition in which T cells and their activation is the essential hinge in the pathogenic process. These functional studies have provided the explanation for the genetic association between CD and certain HLA alleles (HLA DQ2 and DQ8). These genetic, molecular and functional studies have permitted the clarification of a powerful Th1 dominated pro-inflammatory response that characterises the small intestine of active CD patients. Despite this unassailable set of information and reports there are some intriguing points that have been raised by a series of studies which have indicated that CD is not only defined by an aberrant prolamine-induced activation of the adaptive immune system. New evidence and re-assessments of old studies, point to a more complex pathogenic cascade, which may help to unravel some of the residual obscure points of CD pathogenesis. Here, we outline the current concepts that indicate a direct involvement of the adaptive immune system and we discuss all the evidence supporting a direct activation of the innate immune system by fragments of prolamines, which are not recognized T cell epitopes and how they could influence CD. The gliadin-induced activation of the 'innate' immune system might also have a significant role in the induction and persistence of many CD complications and most definitively for the most aggressive one, namely mucosal T cell lymphomas. We further suggest a novel way to harness the unwanted immune response to toxic prolamine, and thus indicate new potential therapeutic strategies to treat or at least control CD.

Keywords: Innate immunity; Celiac disease; Gluten/gliadin; IL-15

1. The adaptive immune system plays a central role in celiac disease

In the last few years, a series of elegant studies has underscored the role of CD4+ HLA-Class II restricted T cells in the pathogenesis of celiac disease (CD). This assumption arose from the long established association of the disease with HLA Class II molecules, in particular HLA DQ2 and to a less extent HLA DQ8 (Lundin et al., 1993). With an apparently simple, but well conceived series of studies, Sollid demonstrated the importance of CD4+ T cells (Sollid, 2000). Although in many other 'autoimmune diseases' an association with HLA has been reported (Nepom and Erlich, 1991) investigators working on CD benefit from a well defined environmental trigger: gluten/gliadin. New and more recent studies have provided further insights into the molecular basis of the HLA association, T cell activation and pathogenesis of CD (Shan et al., 2002). These studies have been instrumental in our understanding of the role of tissue transglutaminase (tTG) in generating high affinity gliadin T cell epitopes and why and how these 'neo' epitopes would preferentially bind to HLA DQ2 molecules (Kim et al., 2004). The field of CD has consequently been totally dominated by a pathogenic

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model centred on the adaptive immune system. Indeed genetic (HLA association) and in vitro studies unequivocally supported this theory. Nevertheless, there was increasing evidence which hinted at a more complex pathogenic pattern.

2. The 'established' pathogenic cascade

Gluten/gliadin initiates CD and CD is defined as gluten/gliadin intolerance, but prolamines from other grains can also induce CD. The main and essential lesion induced by gliadin challenge is damage of the small intestine of active CD patients (Maki and Collin, 1997). Such small intestine lesions characterize and define CD patients and indeed their detection is necessary for a correct diagnosis (Maki and Collin, 1997). Therapy in CD is based on the elimination of gluten and related prolamines from the diet, the so-called gluten free diet (Fasano and Catassi, 2001). Thus, we have celiac patients on gluten containing diet (the so-called 'active' celiacs) and celiacs on a gluten free diet (the so-called 'inactive' celiacs). We will discuss later on how even 'inactive' celiacs have specific modification(s) in their intestine which discriminate them from healthy controls as well as other inflammatory responses of the gastrointestinal tract. In recent years, CD has assumed a pre-eminent role in gastrointestinal disorders because of its high prevalence of around 1 in 100, (Fasano and Catassi, 2001; Maki et al., 2003). This has been a dramatic change from previous estimates which suggested a much lower prevalence. Previous lower prevalence was caused by the fact that relatively few of the active patients presented with classical symptoms, the so-called tip of the iceberg (Maki and Collin, 1997). Another reason, and possibly the main, for the present prevalence of CD are epidemiological studies utilising sensitive new serological tests based on the detection of antibodies to tissue transglutaminase (Dieterich et al., 1997). This has been a revolutionary assay as it has permitted the true prevalence of the disease to be uncovered and helped to unmask even the 'hidden' celiac patients which, as untreated CD, might develop a string of complications which can be life threatening (Loftus and Loftus, 2002). A gluten free diet is thus the only therapy that can be provided to celiac patients. However, a gluten-free diet it is not a true therapy, as patients on a gluten-free diet will relapse as soon as gluten is reintroduced in the diet. It is also sometimes difficult to follow a completely gluten free diet (i.e. poor compliance) and some patients continue to include gluten in their diet. More disturbing is the fact that poor diet compliance is often unintentional because gluten may contaminate foods that are considered to be gluten free foods. Therefore, although CD is usually a common and benign condition, in some cases it may become quite aggressive and untreatable. Therefore, there is no 'proper' therapy.

The HLA association with CD was first demonstrated nearly 30 years ago and more recent studies have indicated that CD is strongly associated with HLA DQ2 (up to 90% of celiac patients express these alleles). The few non HLADQ2 celiacs are HLA DQ8 (Sollid, 2000). However, CD is a complex polygenic disease in which several other genes must also be involved and a series of large genetic studies have addressed this point. Moreover up to 35% of the 'normal' population express HLA DQ2 and not all of these individuals are celiacs, stressing the point that HLA is important, but obviously not the sole 'gene' involved. Because of the functional role of HLA in genetic restriction for antigen presentation to T cells, the well known accumulation of activated CD4+ T cells in the small intestine lamina propria of active celiac patients, a logical approach has been to study the responsiveness of CD4+ T cells to gluten. This option was a natural strategy to follow as diseased small intestinal biopsy tissue from patients with CD is relatively easy to obtain. These small fragments can also be cultured ex-vivo and exposed to gluten or gliadin peptides resulting in tissue damage due to specific mucosal CD4+ T cell activation (Maiuri et al., 1996, 1998).

From the perspective of an experimental immunologist, CD possessed the entire cast for the study of CD4+ T cell pathogenic responses. Indeed there is a well defined HLA association, the triggering antigen, gluten and in particular gliadins are known and sequenced and there is abundant availability of a large number of infiltrating in vivo activated CD4+ T cells in the target tissue. All the evidence and results obtained to date indicate the importance of the adaptive immune system in causing CD. However, it must be remembered that the T cell component of the adaptive immune system is not the only player involved. Indeed a serological response, clearly B cell controlled and CD specific, was described in the early seventies (Seah et al., 1971). This autoantibody response was first defined as anti-reticulin and then anti-endomysium (EMA) (Korponay-Szabo et al., 2003; Maki et al., 1991, 1995) to indicate a 'poorly' defined reactivity to an extracellular matrix component of the small intestine. Interestingly, reactivity was detected not only in the small intestine but also in other tissues and importantly in tissues from other species indicating that the recognized 'antigen or antigens' was widely distributed and well preserved among different species (Amara and Husebekk, 1998). In 1997, Schuppan and colleagues reported that the autoantigen recognized by the celiac specific autoantibodies was transglutaminase type II a tissue transglutaminase tTG (Dieterich et al., 1997). These anti tTG antibodies and the complementary EMA are highly specific for celiac patients with both sensitivity and specificities approaching 95% (Dieterich et al., 1998; Sulkanen et al., 1998). The availability of such a specific serological marker has permitted the more recent epidemiological studies which have redefined the true prevalence of CD. The most surprising finding, however, relating to tTG has been the discovery that tTG is not only the main autoantigen in CD but also the enzyme that modifies toxic prolamine. Though T cell reactivity to gliadin was long suspected and reported (Lundin et al., 1993), it was puzzling that these epitopes showed a relatively poor binding affinity for HLADQ2. These results implied that in some way

gliadin/gluten was modified in situ to increase its potential to stimulate T cell activation. Only in the late eighties was it possible to demonstrate that tTG was the enzyme able to modify, via a deamidation, some key glutamines (Q) to glutamic Acid (E) transforming a non-toxic fragment of gliadin into dangerous stimulators of CD4+ T cells (Molberg et al., 1998; van de et al., 1998). tTG thus assumed a new role not only as the autoantigen of the adaptive and disease specific immune response but also as the 'modifier' of the toxic fragments of gliadin and related prolamines. In doing this tTG was able to unmask the dominant gliadin T cell epitopes. This is at the basis of the cryptic hypothesis so important in the induction of autoimmunity as described in human autoimmune diseases (Quaratino et al., 1996, 2004). Post-translational modifications are an alternative way to unmask or reveal pathogenic epitopes which have been described in an animal model of autoimmunity (Michaelsson et al., 1994). Post-translational modification can also alter antigen processing and the T cell activation controlling the activation of potentially pathogenic T cells (Manoury et al., 2002; Anderton et al., 2002).

The pathogenic cascade of celiac disease focussing on the central role of the adaptive immune system was further clarified when it was reported that a 33 amino-acid stretch of α-gliadin possessed such immunodominant characteristics. This peptide is apparently extremely resistant to intestinal digestion and is readily available for T-cell recognition and activation (Shan et al., 2002), which may explain why this fragment of α-gliadin is recognized by practically all celiac patients (Shan et al., 2002). Remarkably, this relatively short fragment of gliadin contains up to six immunodominant epitopes including the one originally described by Sollid's group (Arentz-Hansen et al., 2000).

3. Is the adaptive immune system alone involved in celiac disease?

So many studies support the central role of the adaptive (T cell) recognition of gluten/gliadin and the validity of this scenario has not been seriously questioned. However, even if the local activation of pathogenic CD4+ T cells is well documented, not all the pieces of the puzzle are yet known. There are several 'gluten-induced modifications' which are not related to CD4+ T cells or to B cell activation. For instance, there are T cells with a unique functional phenotype which differentiates them from typical T cells and these appear to be more associated with innate rather than adaptive responses. This second group of T cells are the intraepithelial lymphocytes (IEL) which are heavily involved in the pathogenesis of CD (Kutlu et al., 1993). These cells are phenotypically and functionally different from the lamina propria CD4+ gliadin specific T cells. They contain a mixture of TCR γδ+ T cells, they are not CD4+ and at least a subgroup express CD94 (Maiuri et al., 2001). A few reports have addressed the role of these cells and recent studies have suggested a role which will be discussed in the following section. A role for IELs has been considered in the past but different hypotheses have been proposed without any clear consensus. Indeed they have been considered to be factors favouring or controlling epithelial damage (Jabri et al., 2000). It has been hypothesised that IEL could induce epithelial death via the engagement of FAS the foremost death receptor which is known to be expressed by epithelial cells (Maiuri et al., 2001). It has also been suggested that they could produce epithelial damage via the release of perforin (Ciccocioppo et al., 2002). An interesting hypothesis is based on the recognition of epithelial cells expressing Class I like molecules which may be upregulated in stressed epithelial cells (Bauer et al., 1999). This hypothesis has recently gained further support from two-elegant studies which have addressed in detail the role of MICA and its recognition by NK2GD (Hue et al., 2004; Meresse et al., 2004). These papers have indicated how the innate immune system and in particular IL-15 a typical cytokine of the innate immune system might be involved in activating IEL in CD. We and others have previously reported that IEL migration and activation at the level of the intraepithelial compartment is controlled by the local release of IL-15 (Jabri et al., 2000; Maiuri et al., 2000). We have championed IL-15 as a key cytokine in CD as it can control different aspects of CD with particular reference to the epithelia (Maiuri et al., 2000).

It has to be emphasised that IL-15 release will play a series of functions which are not only associated with epithelial stress but modify the tone of the adaptive immune response. In particular, it appears to contribute to the marked pro-inflammatory mileau observed in the small intestine of celiac patients. In CD a powerful Th1 response is detected with specific and prompt IFN-γ release in small intestine biopsies of CD patients (Forsberg et al., 2002). That IFN-γ is important has been suggested by a series of studies showing that inhibition of this cytokine has clear protective functional effects (Przemioslo et al., 1995). The main force behind a Th1 dominated response is considered to be IL-12 but this cytokine is not detected in CD, and thus can be excluded from the list of potential conductors of the Th1 dominated response (Nilsen et al., 1998). Others cytokines, such as IL-18, which have been reported as initiators of a Th1 response have not been unambiguously described in CD. IL-15 may also prime towards a Th1 response (Fehniger and Caligiuri, 2001), and thus IL-15 secretion prior to activation of the adaptive immune system would perfectly fit the requirements of the primary factor in CD. IL-15 is implicated in the migration and expansion of IEL, which are abnormally increased in CD (Maiuri et al., 2001b), and may further aid the induction of stress HLA Class I like molecules in epithelial cells. More importantly it could direct the Th1 dominated gliadin specific T cell activation and has anti-apoptotic activities which would further benefit the survival of the 'pathogenic' T cells.

In the context of different pathologies of the gastrointestinal tract it has been reported that inflammation permits unwanted survival of mucosal lymphocytes (Bu et al., 2001). The surprising finding is that rapid lymphocyte turnover is observed in normal mucosa whilst inflamed tissue has a much

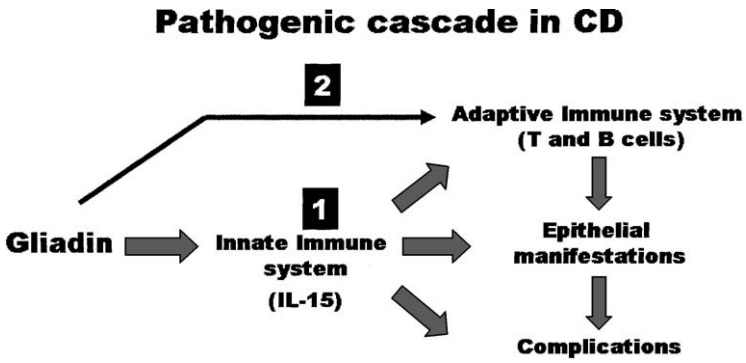

Fig. 1. Model of the pathogenic cascade of celiac disease (CD) illustrating a potential role of the innate immune system. The innate (1) immune response to gluten precedes and defines the type and intensity of the adaptive (2) immune response to gluten.

reduced rate equilibrium being maintained by a modification of the apoptotic rate of resident T cells. If this has a potential benefit when fighting infections it may permit the survival of bystander T cells which could initiate an autoimmune response. IL-15 could have this role but these authors never pinpointed the type of cytokine involved. We have demonstrated that IL-15 displays anti-apoptotic properties in CD (Londei et al., 2003). So IL-15 may be involved in the primary epithelial stress with the induction of HLA Class I like molecules allowing the expansion and migration of IELs. Moreover, it may help in the induction of TH1 response and ultimately protect pathogenic CD4+ T cells from death. It is, therefore, increasingly apparent that IL-15 could have a unique role in the pathogenic cascade of CD.

If IL-15 has such an important role in CD, we should readdress the general belief that in CD the whole pathogenic cascade is controlled and dominated by the activation of the adaptive immune system. IL-15 is in fact, a cytokine only produced by 'innate' cells, mainly monomyeloid but also importantly by epithelial cells. However, it is not produced by T or B cells (Fehniger and Caligiuri, 2001). IL-15 appears to be uniquely placed in celiac disease as it is rapidly induced after gliadin exposure and only in celiac patients (Maiuri et al., 2000). We have recently explored which gliadin peptides induce such a rapid IL-15 release/redistribution (Maiuri et al., 2003). We use the term release/redistribution as this cytokine is mainly controlled at the post-transcriptional level and is often detected on the surface of cells in the absence of typical release (Waldmann et al., 2001). This peculiarity of IL-15 equates to a significant difficulty in the detection of this cytokine as ELISAs often fail to detect it and PCR is ineffective as mRNA is readily detected in unstimulated samples (Fehniger and Caligiuri, 2001; Waldmann et al., 2001). The only option is these cases, as in CD, is the necessity to prove a biological effect of IL-15 inhibition. This can be achieved via specific neutralizing antibodies (Maiuri et al., 2000) or by the soluble α chain of the IL-15 receptor (Ruchatz et al., 1998). Importantly it has to be emphasised that a soluble form of the IL-15 α chain receptor is normally shed by many different types of cell in an inflammatory environment due to the action of specific metallo-proteinases (Mortier et al., 2004; Budagian et al., 2004). All the evidence indicating that IL-15 is a central player in the pathogenesis of CD has suggested that neutralization of this cytokine might hamper the CD pathogenic cascade. The use of organ culture of small intestine biopsies has indeed shown this to be the case (Maiuri et al., 2000).

More interestingly, we have recently demonstrated that different fragments of gliadin can induce activation of the innate immune system specifically in celiac patients (Maiuri et al., 2003). The interesting point of our study was the fact that the innate gliadin peptide was able to prime the adaptive response to the immunodominant gliadin peptides as illustrated in Fig. 1.

CD disease as indicated above is often a relatively mild disease but, albeit rarely, it sometimes expresses itself as a condition called 'refractory sprue' (Cellier et al., 2000), in which a monoclonal expansion of the IEL population is evident and can be considered a precancerous lesion preceding frank mucosal lymphoma (Cellier et al., 2000). IL-15, by controlling IEL, has been described as the cytokine that is clearly involved in celiac sprue and mucosal lymphoma associated with CD. In these patients, therefore we have a situation in which even a strict gluten free diet may not be sufficient in treating refractory sprue and those suffering from mucosal lymphoma (Corrao et al., 2001). It has to be stressed that these patients (refractory sprue) have all the characteristics (HLA genotype, serological profile) of CD patients.

4. Are there alternative options to treat CD?

There are a series of potential options in devising new treatments of CD. We can focus on the adaptive response to gliadin and associated prolamines and try for instance, to develop a powerful anti-inflammatory response to gliadin. It would be possible therefore to define whether a 'natural' anti-inflammatory response, dominated by responsive T cells producing immunoregulatory cytokines such as IL-10 is present in normal HLA-DQ2 or -DQ8 individuals. Research in the area of T regulatory cells (CD4+,

CD25+ or Tr1) is currently flourishing and we may expect a series of studies exploring the viability of such an option in CD. Other cytokines and pathways should also be considered.

The most obvious approach is neutralization of gliadin and related prolamines which can occur on 'complete' digestion of the toxic gliadins. Thus, enzymes which completely digest gliadin and related prolamines would remove the toxicity, and it should be possible to use enzymes such as propyl endopeptidase from *Flavobacterium meningosepticum* as recently suggested (Shan et al., 2002). The mode of delivery is likely to be complex but it should be considered only when toxic prolamines are introduced, and therefore it will not represent a real cure.

A more exciting possibility is based on the targeting of the primary response to the prolamine, in which CD4+ resident T cells play a critical role (Maiuri et al., 1996, 1998). Alternative strategies may also prove highly effective. As mentioned above there is a clear involvement of the IEL population (Ciccocioppo et al., 2002; Jabri et al., 2000; Kutlu et al., 1993), which appears to be largely unrelated to the T CD4+ gliadin specific T cell response. The IEL population is upregulated even after a long period of strict gluten free diet and appears to be involved with the epithelial destructive response. IELs are very responsive to IL-15 and this cytokine regulates their activation and expansion (Jabri et al., 2000). IL-15 has been the target of our research and we and others have demonstrated upregualtion of IL-15 in CD. The use of organ culture of small intestine biopsy has been essential to indicate that IL-15 neutralization might have a specific therapeutic value for CD (Maiuri et al., 2000). IL-15 inhibition has been targeted in other pathologies and in animal models (Ruchatz et al., 1998) and has become a potential target for many autoimmune related pathologies (McInnes and Gracie, 2004). In view of our results, together with the literature on IL-15 and IEL in CD, we suggest that IL-15 neutralization might become an attractive option for the management of CD. The specificity of the IL-15 receptor complex is provided by the α chain of the IL-15 (Waldmann et al., 2001) as the other two chains (β and γ) are also shared with the IL-2 receptor complex (Waldmann et al., 2001).

Specific, local IL-15 neutralization could represent an ideal way to control the IEL population. But there is another important aspect of IL-15 neutralization which has to be considered. IL-15 inhibition allows the induction of apoptosis of T cells in the lamina propria and many of these cells are the CD4+ pathogenic gliadin specific T cells. This means that release of soluble IL-15 α-chain may delete a large section of mucosal CD4+ gliadin specific pathogenic T cells that are prone to apoptosis. Another potential benefit of neutralization of IL-15 is possible in refractory sprue (Cellier et al., 2000) which is characterized by an uncontrolled expansion of IEL. There is increasing evidence that IELs are sensitive to IL-15, and therefore neutralization of this cytokine via a series of approaches may further benefit celiac patients (Londei et al., 2003).

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