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Immunoregulation in celiac disease

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Abstract

Celiac Disease (CD) is a T-cell mediated immune disease, in which gluten-derived peptides activate lamina propria effector CD4+ T cells. While these effector T cell subsets produce proinflammatory cytokines, which cause substantial tissue injury in vivo in CD, recent data suggest that additional subsets of CD4+ T cells exist with suppressor functions. These subsets include type 1 regulatory T cells (Tr1) and CD25+CD4+ regulatory T cells expressing the master transcription factor Foxp3, that have important implications for disease progression. In this review, we provide an overview of the current knowledge about the immune-mediated effects of cytokines produced by suppressor Treg cells in CD and how the regulatory properties of these cells could be influenced.

Introduction

The ability of the immune system to distinguish between self-antigens and nonself-antigens, and between harmful and innocuous foreign antigens, is critical to the maintenance of immune homeostasis. Failure to maintain tolerance to self-antigens or innocuous antigen results in the development of autoimmune or allergic disease, respectively. To achieve this state of immune tolerance, the immune system has evolved a variety of mechanisms. These include relative T cell inactivity or T cell anergy [1,2], T cell depletion by apoptosis [3,4] and active immune suppression by regulatory T cells (Treg) [5,6]. Several Treg subsets are involved in immune tolerance [7]. These subsets include natural Treg cells expressing the forkhead box P3 (Foxp3) transcription factor which are selected in the thymus and antigen-induced Foxp3+ cells, generated in the periphery [8]. Tr1 cells, that downregulate naive and memory T-cell responses upon local secretion of IL-10 and transforming growth factor-β (TGF-b) [9], and TGFβ-producing Treg cells (Th3) [10], are other important subsets that possess regulatory properties, both induced in the periphery. TGF-β and IL-10 play an essential role in the differentiation of Foxp3+ and Tr1 cells, respectively [11, 12].

Different mechanisms of Treg-mediated suppression have been described, mostly on the basis of *in vitro* suppression assays. From a functional perspective, the various potential suppression mechanisms of Treg cells can be grouped into four basic 'modes of action': suppression by inhibitory cytokines, suppression by cytolysis, suppression by metabolic disruption, and suppression by modulation of dendritic-cell (DC) maturation or function. Inhibitory cytokines' include IL-10, IL-35 and TGF-β. 'Cytolysis' includes granzyme-A or B-dependent and perforin-dependent killing mechanisms. 'Metabolic disruption' includes high affinity IL-2 receptor α (CD25)-dependent cytokine-deprivation-mediated apoptosis and cyclic AMP (cAMP)-mediated inhibition. 'Targeting dendritic cells' includes mechanisms that modulate DC maturation and/or function such as MHC-class-II-mediated suppression of DC maturation, and cytotoxic T lymphocyte antigen-4 (CTLA4)–CD80/CD86-mediated induction of indoleamine 2,3-dioxygenase (IDO), which is an immunosuppressive molecule, by DCs [13].

Celiac disease (CD) is a autoimmune-mediated enteropathy triggered by the ingestion of gluten-containing grains (including wheat, rye and barley) that occurs in about 1% of the population. The characteristic features of CD histology have always been considered to be a villous atrophy, crypt cell hyperplasia, and increased number of intraepithelial lymphocytes (IELs).

An important feature of CD is its strong genetic association with HLA class II genes: more than 90% of patients carry the HLA-DQA*0501/DQB*0201 (DQ2) alleles, whereas the majority of DQ2-negative patients are DQ8-positive (DQA*0301/DQB*0302). The strong genetic association with HLA molecules is strengthened by the finding that gluten-derived peptides are recognized by celiac intestinal $CD4^+$ T lymphocytes when presented by the disease predisposing DQ2 or DQ8 molecules. In fact it is generally accepted that a recognition of gluten peptides in association with HLA Class II molecules by Th1 T cells secreting proinflammatory cytokine leads to the overt CD lesions at the intestinal level [14].

At the same time, an enhanced expression of anti-inflammatory cytokines, such as IL-10, has also been observed in CD. In fact, in untreated CD patients the levels of IL-10 are higher, but the ratio IL-10/IFN γ is significantly lower in comparison with inflamed non-celiac, control, and treated CD mucosa [15,16]. This apparent paradoxical milieu of both pro-inflammatory and suppressive cytokines strongly suggests that regulatory mechanisms might operate to counterbalance the gliadin-triggered, abnormal immune activation in untreated CD [16]. Importantly, we have observed that celiac intestinal mucosa harbors two subset of Treg cells, known as type 1 regulatory T cells (Tr1) and Foxp3+ Treg cells , which, through the release of both IL-10 and TGF-β, inhibit the pathogenic response to *in vitro* gliadin challenge [17,18]. Recently, we found that intestinal Treg cells can be impaired in their suppression capacity by IL-15 in active CD [18].

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Herein, we discuss the potential role of Treg cells and suppressive cytokines in controlling the gluten-dependent inflammation in celiac intestinal mucosa and how signals received from the tissue environment could influence the regulatory activity of such cells.

Type 1 regulatory T cells in CD

Experiments performed to induce therapeutic tolerance via the gut in various autoimmune disease models have relied on a bystander effect of stimulated T cells which,through immune deviation, preferentially have secreted down-regulatory cytokines, particularly TGF-β [19]. The gut has been suggested to harbor T cells with a propensity for secretion of TGF-β (so-called Th3 cells), which appear to be particularly resistant to apoptosis, [20] but this subset has not been clearly identified in humans. Another regulatory T-cell subset (Tr1) with a remarkable propensity for IL-10 production has been identified both in the murine and human gut [21,22].

Interleukin (IL)-10 is an important immunoregulatory cytokine which acts on antigen presenting cells *via* inhibition of cytokine synthesis and expression of costimulatory and MHC class II molecules [23–26]. In addition, IL-10 directly interferes with T cell proliferation and differentiation [27,28]. Activation of human $CD4^+$ T cells with allogeneic antigen presenting cells in the presence of IL-10 results in a long lasting antigen specific unresponsiveness of T cells [29]. Furthermore, IL-10 induces differentiation of type 1 T regulatory (Tr1) cells which are able to suppress the Th1 immune response *in vivo* and *in vitro* through secretion of IL-10 and TGF-b [30,31]. Although under healthy conditions gut lamina propria T cells (LPT) spontaneously secrete high levels of IFN-g [32] it has been shown that LPT can also release high amounts of IL-10 *in vitro* [33]. In explant cultures of human fetal gut, human recombinant (rh)IL-10 downregulates mucosal T cell activation, metalloproteinase production, and prevents loss of extracellular matrix and mucosal damage induced by pokeweed mitogen or Staphylococcal enterotoxin B [34].

In CD, the role of IL-10 has not yet been fully clarified [35-37]. Increased levels of IL-10 and IFN-γ have been reported in IELs isolated from untreated coeliac duodenal biopsies [16]. However, other cells, such as macrophages, epithelial, and dendritic cells may also contribute to increased levels of IL-10. The fact that the ratio between mRNA levels for IL-10 and IFN-γ was significantly lower in untreated and inflamed CD mucosa in comparison with other enteropathies strongly suggests that even these high levels of IL-10, which presumably reflect a compensatory antiinflammatory pathway, are not sufficient to suppress the overwhelming Th1 mediated response in active CD.

The organ culture system of the treated celiac intestinal mucosa is an experimental system to explore the immunological events triggered by the contact of the antigen gliadin with the jejunal mucosa. Changes suggesting an activation of T cell immunity have been shown at the lamina propria level, including increased density of monuclear cells expressing IL2 receptor or enhanced expression of costimulatory molecules or adhesion molecules. As far as cytokines are concerned, a strong enhancement of mRNA expression for g- interferon and IL2 has been observed [38].

By using such system we found that the addition of exogenous IL10 can potently downregulate Th1 mediated immune responses to gliadin. IL-10 mediated its effects in terms of the reduced densities of activated T cells, reduced expression of CD80/CD86 costimulatory molecules and mRNA for inflammatory cytokines [15].

In addition to the organ culture system, gliadin-specific T-cell lines and T-cell clones obtained from intestinal biopsies have provided great support in the investigation of immuno-pathogenesis of CD [39]. In fact, many of the gluten T-cell stimulatory sequences have been identified thanks to the availability of stable T-cell lines and T-cell clones raised from intestinal mucosal tissues [40]. Based on the findings that rhIL-10 induced long lasting antigen specific unresponsiveness of antigen specific $CD4^+$ T cells [29,30], we investigated whether rhIL-10 could specifically downregulate Th1 mediated immune responses in treated CD mucosa challenged with PT-gliadin *in vitro*. Mucosal explants from treated CD patients were cultured for 24 hours with PT-gliadin in the presence or absence of rhIL10. We found that the production of IFN-γ upon gliadin stimulation is absent at 3 weeks after the isolation of gliadin-specific T-cell lines (iTCLs) from biopsies cultured with gliadin in presence of IL10, suggesting that IL-10 treatment induces an anergic state of mucosa-derived, gliadin-reactive T cells [15]. Moreover, we found an increased frequency of IL-10 producing cells in TCLs obtained from explants cultured in the presence of rhIL-10. Therefore, we provide the first evidence for an immunoregulatory effect of IL-10 on gliadin dependent T cell activation in treated and untreated CD mucosa. IL-10 acts by interfering with antigen presentation and results in induction of hyporesponsive gliadin specific T cells.

Since IL-10 is involved in the differentiation of Tr1 cells [12], in another series of experiments we looked at the presence of these cells in our iTCLs. We found that when the functions of IL-10 and TGF-β (the two main Tr1 cytokines) are blocked using specific neutralizing antibodies, an increased immune activation to gliadin stimulation is observed in the vast majority of iTCLs generated. These data suggest the existence of endogenous anti-inflammatory mechanisms in celiac disease mucosa to control local gliadin-induced inflammation. Subsequently, the cell cloning of gliadin-specific iTCLs revealed that the celiac intestinal mucosa harbors gliadin-reactive Tr1 cells that show a low proliferative rate to gliadin stimuli, but suppress pathogenic T cells through the release of both IL-10 and TGF-β [17].

Collectively, these *ex vivo* and *in vitro* results suggest that gliadin-specific Tr1 cells differentiate *in vivo* most likely as a consequence of the marked IL-10 production in inflamed celiac disease mucosa [Box 1].

Box 1 | Type 1 regulatory T cells in CD

Tr1 cells regulate immune responses through the secretion of the immunosuppressive cytokines IL-10 and transforming growth factor-b, and they suppress T-cell responses [9]. IL-10 is absolutely required for the differentiation and function of mouse Tr1 cells. In the untreated intestinal mucosa, concomitantly with the pro-inflammatory response, high amounts of the anti-inflammatory cytokines IL-10 and TGF-β are also produced. Interestingly, gliadin-specific Tr1 cell clones from intestinal mucosa of celiac patients on gluten free diet were isolated. These gliadin-specific Tr1 cell clones produced IL-10 and TGF-b, and suppress the Th1 patogenic T cells [15,17]. These findings open a new perspective for immunotherapy in CD by boosting in vivo the number of Tr1 cells or cell therapy with ex vivo generated Ag-specific Tr1 cells.

Foxp3+ T regulatory cells in CD

Foxp3 has been proposed as a master key regulator for Treg, required for their differentiation, maintenance, and suppressive functions. Foxp3 induction in natural Treg occurs *in vivo* during

thymic differentiation, under the influence of relatively high avidity interactions of the TCR with self-antigens [42]. Foxp3 can also be induced post-thymically, in conventional mature T lymphocytes when they are activated *in vitro* or *in vivo* in the presence of the cytokine TGF-β [43-45]. Tr1 cells, on the other hand,are a more discrete population of Treg that are induced in the periphery which, to date,lack a define cell surface signature. Furthermore, Foxp3 is not required for Tr1 cell induction or function since suppressive Tr1 cells can be generated or isolated from peripheral blood of patients with immunedysregulation polyendocrinopathy enteropathy X- linked (IPEX), a disease condition due to Foxp3-mutations, even in those patients with complete deletion of Foxp3 [46]. Therefore, Tr1cells and Foxp3+ Treg in humans are distinct subsets of cells with regulatory activity that cooperate in promoting and controlling tolerance *in vivo*.

We and others reported an increase of Foxp3+ cells in untreated intestinal mucosa compared to non-inflamed mucosa (either treated or control subjects) [18,47-50]. These data strongly supports the hypothesis that the immune system is actively trying to downregulate ongoing inflammation either through the rapid redistribution of Treg cells from the circulation to the inflamed site, or through the local proliferation of these regulatory cells.

In humans, the correlation between Foxp3 expression and suppressive capacity is not as clear as in the murine system. Infact, recently it has been shown that expression of Foxp3 does not exclusively occur in $CD4^+$ $CD25^+$ Treg cells, as in humans it can also be transiently induced in activated $CD4^+$ $CD25^-$ T effector cells, which do not express Foxp3 in the resting state [51,52]. This means that Foxp3 expression in human T cells does not always directly correlate with suppressive capacity [53,54]. Therefore, to ascertain whether in active CD patients the observed Treg cells are indeed suppressive, we isolated $CD4^+$ $CD25^+$ cells from biopsy samples and tested their suppressive capacity in an *in vitro* coculture assay. Importantly, we showed that such cells are able to exert their regulatory effects *in vitro* in terms of inhibition of proliferation and IFN- γ secretion [18].

These regulatory effects may also operate *in vivo*. Neverthless, despite the increased frequency and suppressive activity, Treg cells fail to control the development of the inflammation in the small intestinal mucosa with active CD. It is possible that the suppressor capacity of these cells may be abrogated *in vivo* or it is insufficient to counterbalance the strong proinflammatory response.

Many factors may interfere with the function of Treg cells. It is relevant to know that IL-15, a potent pro-infl ammatory cytokine overexpressed in the intestine of patients with active CD, interferes with immune regulation, functioning on TGF-β activity, which is required to maintain the regulatory function of Treg cells [55], thus contributing to the loss of intestinal homeostasis and promoting chronic inflammation [56]. Subsequently, it was also found that IL-15 renders effector T cells resistant to suppression by Treg cells [57,58]. Therefore considering these results, we evaluated in CD the effects that IL-15 exerts on the suppressive function of Treg cells.

We have shown, in active CD patients, that IL-15 impairs the functions of Treg cells making T effector cells refractory to the regulatory effects of $CD4^+$ $CD25^+$ T cells, in terms of proliferation and production of IFN-γ (18).

Similarly, IL-21 has been suggested to abrogate Foxp3+ Treg function [59]. Recently, it has been shown that IL-21 expression is increased in the intestinal mucosa of patients with active CD [60].

Therefore the inhibitory effect of IL-15 on transforming growth factor (TGF)-β signaling and of IL-15 and IL-21 on the response of effector T cells to Foxp3 regulatory T cells may perhaps participate in the induction of autoimmune diseases in CD. Therapies aimed at neutralizing these cytokines may offer new therapeutic opportunities to restore gut homeostasis [Box 2].

Box 2 | Foxp3+ regulatory T cells in CD

T regulatory cells, characterised by the expression of the transcription factor Foxp3, play a key role in maintaining immune homeostasis by suppression of the activity of effector T cells. Foxp3+ regulatory T cells are increased and functionally efficient in active CD (18). Expansion of this subset, proportional to the intensity of local inflammation, could play a role in the negative feedback loop of T-cell activation. Nevertheless, the function of Foxp3+ cells might be substantially limited through IL-15, a cytokine overexpressed in the intestinal mucosa of patients with active CD, thus restricting the control of the ongoing immune response to gliadin and consequent inflammation [18].

Therapies aimed at neutralizing these cytokines might not only decrease bystander T-cell activation but also reconstitute the suppressor function of Foxp3 regulatory T cells.

Conclusion

In untreated celiac mucosa beside the massive production of pro-inflammatory cytokines, an enhanced expression of anti-infl ammatory cytokines, such as IL-10, has also been observed, suggesting that an immunoregulatory mechanisms may operate to control inflammation.

Two subset of regulatory T cells, Tr1 and Foxp3+ T cells, have been identified in celiac intestinal mucosa. Nevertheless, signals received from the tissue environment, as IL-15, could influence the regulatory activity of such cells, suggesting that these cells, even if recruited may be ineffective to downregulate the massive inflammatory response driven by gliadin.

As Treg cells exist naturally in the human gut mucosa and maintain intestinal homeostasis, using methods to enhance their numbers and/or function is a possibility worthy of pursuit as cellular therapy to re-establish tolerance to gluten in patients with CD.

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