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Chapter

Immune Checkpoints as a Novel Source for Diagnostic and Therapeutic Target in Celiac Disease

Isabel Torres, Miguel Ángel López Casado, Teresa Palomeque and Pedro Lorite

Abstract

Celiac disease, as an autoimmune disorder, is a disease which appears in sensing and immune reaction responses to gluten. It has been confirmed that both genetic and environmental factors are involved. CD is strongly associated with the HLA alleles DQB1*02 (serological DQ2) or DQB1*0302 (serological DQ8). These HLA alleles are necessary but not sufficient for the development of CD and non-HLA risk genes also contribute to disease susceptibility. Several studies have identified linkage or association of CD with the 2q33 locus, a region harboring the candidate genes CD28, CTLA4 and ICOS, important immune checkpoints regulators of T-cell activity. Immune checkpoints are crucial to maintain self-tolerance and protect self-tissue from damage during an ongoing immune response.

Keywords: immune checkpoints, celiac disease, PD1, PDL, HLA-G, CTLA4, IDO, tryptophan

1. Introduction

Celiac disease is a unique autoimmune disorder in that the key genetic components (HLA class II genes DQ2 and/or DQ8) are present in almost all patients, the autoantigen is known (tTG), and, most importantly, the environmental trigger is known (gluten) [1–5]. The HLA-DQ molecules predispose to disease by preferential presentation of gluten antigens to CD4+ T cells [6–8]. These genotypes are necessary for the development of the disease, but they are not the only ones responsible, since these genes are present in the population, and only 1% develop CD [9]. Furthermore, in recent years, other areas of the genome outside the HLA region have also been identified that could influence susceptibility to CD, many are related to immunity, especially with T-cell and B-cell function [10, 11].

Gluten ingestion by patients with CD leads to a cascade of inflammatory reactions and eventually to the hallmark small-intestinal lesion. The most important consequence is reduced nutrient uptake characterized by $CD4^+$ T-cell activation, increasing numbers of intraepithelial lymphocytes with partial to total intestinal villus atrophy [12–15]. A common feature of gluten-derived epitopes is the presence of multiple proline and glutamine residues that are selectively deamidated by tTG.

The passage of immunogenic peptides to the lamina propria stimulates specific CD4 + T lymphocytes when they occur together with HLA-DQ2/DQ8 molecules, after having a modification by tissue transglutaminase (TGt). Proinflammatory cytokine responses are activated and mechanisms causing mucosal alteration. Activation of these gluten-reactive CD4 + T cells lead to a pro-inflammatory response dominated by IFN-γ production [16–18].

The response of CD4 + T cells to post-translationally modified gluten and highly disease specific B cells to deamidated gluten and transglutaminase 2 (TG2) autoprotein are present in the pathogenesis in CD [12]. When immunogenic gluten peptides cross the intestinal lumen, they can trigger an innate and adaptive immune response, leading to the development of clinical and histological manifestations of CD [19].

The immune homeostasis has to be precisely maintained in a physiological state, through a balance of costimulatory (*e.g.* CD28) and coinhibitory (*e.g.* CTLA-4 or PD-1) immune signals known as "immune checkpoints". Immune checkpoints are essential for maintaining self-tolerance, protecting tissues from damage caused by the immune system, and providing protective immunity [20]. An imbalance in immune homeostasis can lead to costimulation and the upregulation of T-cell activation in autoimmune diseases [21].

During the normal activation state, $CD4^+$ and $CD8^+$ T cells express multiple immune checkpoint molecules, and some of them also serve a costimulatory function of T cells activation. T cells obtained from individuals with autoimmune conditions have enhanced expression of these molecules that represent an activated T cell state. T lymphocytes play a central role in the induction of an effective adaptive immune response and responsible for maintaining immune homeostasis. Signaling through two well-known negative regulators or checkpoints of T cells, CTLA4 and PD1 leads to direct inhibition of T cell responses [20].

The present chapter discuss the role of the immune checkpoints in intestinal tissue homeostasis and tolerance, and speculate how genetic and environmental factors can regulate them in celiac disease.

2. Immune checkpoints

Immune checkpoints play an essential role in the function and regulation of effector T cells (Teff) and regulator T cells (Treg). Immune checkpoint molecules limits excessive T cell-mediated inflammatory responses and in their signaling processes include a series of ligands that are expressed on the membrane of antigen-presenting cells (APCs) transmitting inhibitory signals (**Figure 1**). These molecules employ specific receptor partners expressed by T lymphocytes and drive their activation and differentiation or promote immunoregulatory effects. Dysregulation of these signaling processes has been associated with autoimmunity and chronic inflammation.

Autoimmune diseases are heterogeneous conditions involving breakdown of tolerance and consequent activation of autoreactive immune cells [22]. The failure of immune checkpoints has been described in inflammatory myopathies with the involvement of autoimmune features [23], as well as in diabetes, multiple sclerosis, systemic lupus erythematosus and celiac disease [24]. Inhibitory checkpoint molecules have been considered as new targets in personalized cancer immunotherapy for their potential in multiple cancer types [21, 25]. An active area of research is the analysis of the functions of these checkpoint molecules and its ligands in tolerance and autoimmunity.

The immune system has the difficult dual function of discerning and defending against a variety of pathogens and avoiding self-reactivity. To further control the

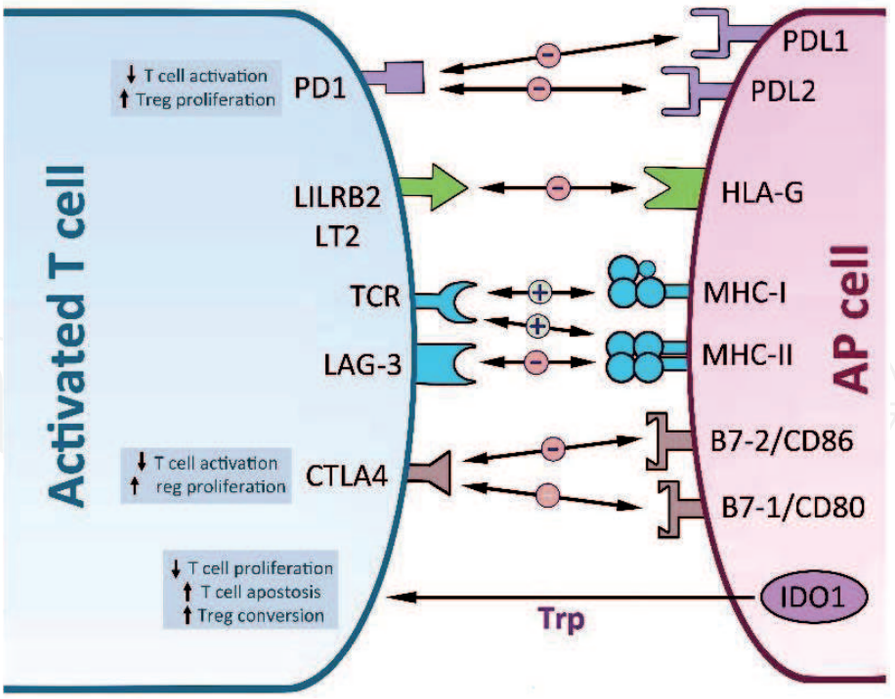

Figure 1.
Schematic representation of immune checkpoint molecules including a series of ligands expressed on the membrane of antigen-presenting cells (APCs), that engage specific receptor partners expressed by T lymphocytes and either drive their activation and differentiation (positive immune checkpoint molecules) or promote immunoregulatory effects (negative immune checkpoint molecules).

development of autoimmunity, multiple mechanisms of peripheral tolerance have evolved, including T-cell anergy, deletion and suppression by Tregs cells. Treg and Teff cells help maintain immune homeostasis through mutual regulation. Loss of homeostatic balance between Teff/Treg cells is often associated with autoimmunity [24, 26].

In this chapter, we will discuss the biology of immune checkpoints, highlight research strategies that may help reduce the incidence of immune related adverse events associated with celiac disease, and also suggest investigational approaches to manipulate immune checkpoints to treat its autoimmune disorder.

2.1 IDO/kynurenine pathway

An important inhibitory checkpoint is now considered to be the tolerogenic mechanism of the enzyme indoleamine-2, 3-dioxygenase (IDO), an intracellular protein involved in the oxidative catabolism of tryptophan (Trp). It catalyzes the conversion of Trp to N-formyl kynurenine via the kynurenine pathway. Depletion of Trp reduces T cell proliferation, whereas the production of kynurenine induces apoptosis of type 1 T helper (Th1) cells and naïve T cell differentiation into Tregs cells [27, 28].

IDO is expressed intracellularly in a constitutive manner in the placenta, epididymis, prostate, esophagus, intestine, colon, cecum, spleen, thymus, lung, brain, and skin [29, 30]. Notably, the morphological features of many IDO-expressing cells closely resemble those of antigen-presenting cells and epithelial cells [29].

IDO expression is inducible by inflammatory stimuli including cytokines and toll like receptor agonists. IDO is expressed in antigen presenting cells, macrophages and dendritic cells, and activation of IDO during the inflammatory response leads to a decrease in local trp levels [27]. These decreased levels have an inhibitory effect on the proliferation of T lymphocytes, directly or indirectly via activation of regulatory T cells [27, 31].

In most cell types, IDO is induced at the transcriptional level in response to specific inflammatory stimuli. IFN-γ is the principal IDO inducer *in vitro* and *in vivo*. Exposure to IFN-γ increases IDO transcription in monocyte/macrophages [32] and dendritic cells [33], fibroblasts [34], epithelial cells [35], smooth muscle cells [36]. Other inflammatory stimuli, such as IFN-α, IFN-β, LPS and cytotoxic T lymphocyte-associated antigen (CTLA)-4, also induce IDO to a lesser degree than that of IFN-γ [37, 38].

A dysfunctional IDO has recently been associated with a specific single nucleotide polymorphism (SNP) and with the occurrence of autoimmune diabetes and multiple sclerosis. The elevated levels of kynurenines that are present contain the proliferation of Teff cells and favor the differentiation of Treg cells [39]. Several genetic variations of the *IDO* gene have been associated with the occurrence and severity of autoimmune/chronic inflammatory diseases; however, the functional relevance of these variations, which mainly affect the intron regions and the promoter portion of IDO, has not been well characterized yet [40, 41].

In celiac disease, mechanisms dependent on tryptophan catabolism may be involved in the regulation of the immune response. Thus, in intestinal biopsies from celiac patients, a high expression of the anti-inflammatory enzyme IDO appears [42]. This increase in IDO levels results in an increase in serum levels of kynurenine in patients with celiac disease, which potentially contributes to intensify inflammation. Likewise, higher levels of kynurenine were found in celiac patients with other associated diseases, such as Down syndrome or autoimmune thyroiditis, contributing to the pathology [43].

Once an autoimmune disorder is established, the presence of chronic inflammation might provoke sustained IDO production and IDO fails to limit immune deregulation under these pathological conditions. Several inflammatory mediators including the most potent, IFN-γ, induce IDO production. Wolf *et al*. [44], have described overexpression of IDO in other Th1-associated chronic inflammatory disease of the gastrointestinal tract, such as Crohn's disease, with increased kynurenine levels and a higher kynurenine/tryptophan ratio. In this pathology has been demonstrated that T helper 1 (Th1)-like cytokines such as IFN-γ and TNF-α are potent inducers of IDO expression.

2.2 HLA-G/ILT interaction is an immune checkpoint

The *HLA-G* gene is a non-classical class I HLA composed of eight exons and seven introns located on chromosome 6 at region 6p21.3, [45]. As a result of alternative RNA splicing, seven isoforms can be formed, comprising four membrane-bound isoforms (HLA-G1, G2, G3, and G4), and three secreted soluble isoforms (HLA-G5, G6, and G7) [46, 47]. Most studies focus on the full-length molecule (HLA-G1) and its soluble isoform (HLA-G5). These isoforms are identical, except HLA-G5 is missing the transmembrane domain.

HLA-G is considered to be an immune checkpoint molecule, a function that is closely linked to the structure and dynamics of the different HLA-G isoforms. The expression of HLA-G can be induced in several conditions, including cancer, transplantation, viral infections, and autoimmune and inflammatory diseases [48–51].

HLA-G mediates its function by binding to receptors on immune cells. The known receptors are leukocyte Ig-like receptor subfamily B member 1 (LILRB1) and member 2 (LILRB2), also known as ILT2 and ILT4, and the killer immunoglobulin-like receptor 2DL4 (KIR2DL4) [52]. ILT2 is expressed by B cells, some subtypes of T cells and NK cells, and all monocytes/dendritic cells. On the other hand, ILT4 is myeloid-specific and only expressed by monocytes/dendritic cells [53].

HLA-G expression and gene polymorphisms have been associated with several disorders [54–56]. HLA-G has an important role in regulating the immune system;

indeed, the molecule is able to inhibit the cytotoxic activity of Natural Killer cells (NK) and T cell-mediated cytolysis (CTL) [57]. HLAG can inhibit the response of alloproliferative CD4+ T cells, proliferation of T and NK cells, and the maturation and function of antigen presenting cells (APC) [58, 59]. In addition, HLA-G has a tolerogenic effect due to its capacity of generating suppressor cells by binding to specific receptors and it can induce apoptosis in endothelial cells [60].

The soluble form of HLA-G is of special interest in celiac disease because its molecule plays an important role in the induction of immune tolerance [61]. In this sense, soluble HLA-G has the function to inhibit the proliferation of activated T cells, and to induce apoptosis of T cells dose dependently, reinforcing the immune inhibitory role of soluble HLA-G capable to be secreted during CD as part of a mechanism to restore the tolerance process towards oral antigens [61, 62]. A potent anti-inflammatory response to gliadin may occur during disease development as a result of the adaptive response in CD. In celiac patients, gluten intake appears to cause an overreaction in intraepithelial T lymphocytes, with uncontrolled production of the HLA-G molecule [61]. This can cause the recruitment of intraepithelial lymphocytes, leading to amplified immune activity and maintenance of intestinal lesions. The increased expression of the soluble form of HLA-G in patients with CD could be part of a mechanism to restore gluten tolerance [61, 62].

Moreover, an association between HLA-G polymorphism and CD has already been described by Fabris et al. [63]. The 14 bp inserted (I) allele and the homozygous I/I genotype were significantly more frequent in CD patients than in healthy controls. The effect of the HLA-G D/I polymorphism is restricted for HLA-DQ2, and not simply due to the presence of linkage disequilibrium with the major known risk factor. In this sense, the risk conferred by HLA-DQ2 alone and that subjects that carry both DQ2 and HLA-G I alleles have an increased risk of CD than subjects that carry DQ2 but not the 14 bp inserted (I) allele [63]. The modulation of the HLA-G transcript stability is especially known for the 14 bp D/I polymorphism, which has been associated with autoimmunity [64–67]. Based on the findings of Torres et al. [61] and considering that the presence of HLA-G SNPs affect the mRNA stability in CD patients, lower basal levels of HLA-G molecule, possibly due to the presence of genetic variations, can increase the risk of celiac disease development. Once that the disease has occurred the organism produces higher levels of soluble HLA-G trying to restore the immune tolerance [61, 62]. Similarly to 5'URR, also 3'UTR presents numerous polymorphic sites that could affect HLA-G transcription and/or translation [68]. By sequencing this region, there are 4 polymorphisms showing some significant associations with CD [64].

In summary, it has been shown that both HLA-G and IDO suppressor molecules are expressed in CD. The expression of these molecules, IDO and HLA-G, would be an essential mechanism to try to restore tolerance towards antigens in the diet [61, 62]. Therefore, the increase in IDO activity reflects an attempt to control chronic antigenic stimulation by downregulating the T cell-mediated autoimmune reaction. IDO and HLA-G could cooperate to suppress the immune response in CD in their active form [61].

López *et al*. [69] showed that suppressive molecules IDO and HLA-G are both expressed in dendritic cells, and these molecules can produce immunosuppression. Besides, IDO was shown to induce HLA-G expression during monocyte differentiation into DCs [69]. IDO and HLA-G share some properties: both have tolerogenic capacity, are highly expressed in human placenta and tumors [70, 71] and their expression can be regulated by the same cytokines (IFN-γ, IL-10) [72, 73]. The effect of IDO on HLA-G cell-surface expression seemed to be dependent on the type of cell studied and is likely to involve posttranslational mechanisms [74]. The inhibition of IDO function with 1-methyl tryptophan in antigen-presenting cells (APCs), which are originally HLA-G cell-surface negatives, increases the levels of

HLA-G1 cell-surface expression, whereas high concentrations of tryptophan caused a loss of HLA-G1 expression in HLA-G1-positive cells [75].

2.3 CD28/CTLA4-B7 pathway

The immune regulatory proteins cytotoxic T lymphocyte antigen (CTLA-4) is important immune regulatory protein collectively referred to as immune checkpoint receptor. CTLA-4 is known to be crucial for tolerance induction in the early stages of the immune response being an important negative regulator of T-cell activation and proliferation, interacting with B7 molecules on antigen-presenting cells [76, 77]. The *CTLA4* gene encodes a receptor involved in the control of T-cell proliferation and mediates T-cell apoptosis. *CTLA-4* is therefore a plausible candidate for a susceptibility gene in diseases with T-cell mediated pathogenesis [77].

The chromosomal region 2q33 contains immunologically important genes, CD28 and ICOS, that has been associated with autoimmune disease, but the exact causal genetic sequence variation has yet to be established in CD [78]. There is good evidence that the *CTLA-4* region on chromosome 2q33 contains a non-HLA susceptibility locus for celiac disease, although its participation may vary according to the geographic origin of the patients [79]. The association of several SNPs in the CTLA4 gene with CD, among them, the functional SNP, CT60, suggested that the CT60 polymorphism influences alternate RNA splicing of CTLA4, resulting in differing ratios of a full-length form, flCTLA4, and a soluble form, sCTLA4 of the protein [78].

High levels of serum soluble CTLA-4 in active celiac patients were found and are related to gluten intake. A positive correlation exists between autoantibodies to tissue transglutaminase, the grade of gut mucosa damage and soluble CTLA-4 concentration [80]. This correlation between the amount of serum sCTLA-4 and the grade of gut mucosa damage strongly suggests a possible immunomodulatory effect of this soluble molecule on cytotoxic T lymphocyte functions. Thus, soluble CTLA4 appears to be related to autoantibody production per se, independently from dietary gluten [80]. Soluble CTLA-4 could play a critical role in modulating the immune response, especially in the early stage. The immunomodulatory effect of soluble CTLA-4 could be involved in the regulation of B cell activation directly or via T helper function modulation [80].

The detection of the spliced/soluble variant from CD patients suggests that the soluble CTLA-4 does not result from a cleavage of the full-length form [80]. The potential genetic associations of several *CTLA-4* polymorphisms to susceptibility to autoimmune diseases have been described, although the relationship between *CTLA-4* polymorphisms and the ability to produce the soluble form is not fully clarified. CTLA-4 is a strong actor in the adaptive response.

2.4 PD1/PDL pathway

Programmed cell death-1 (PD-1) is a well-established immune checkpoint and co-inhibitory regulator critical to the maintenance of immune tolerance. PD-1 through binding to its PD-L1 and PD-L2 ligands, generate inhibitory signals that regulate the balance between immune system activation, tolerance and immunopathology [81]. The PD1 expression has been noted in activated CD4+/CD8+ T cells, a subset of Tregs, B-cells, myeloid DCs, monocytes, exhausted T cells and basal mesenchymal stem cells [82]. Basal levels of expression of ligand PDL1 was observed in mesenchymal stem cells and vascular endothelium. In addition, activated B-cells, DCs and monocytes also express both PD-L1 and PD-L2 [82]. Lower levels of PDL1 expression was reported in unstimulated CD4+/CD8+ T-cells which was increased upon activation.

Cytokines are potent stimuli for PD-L1 and PD-L2 expression. Type 1 and type 2 interferons and TNF-α induce PD-L1 expression in T cells, B cells, endothelial cells, and epithelial cells [83]. IL-2, IL-7, and IL-15 cytokines increase PD-L1 on human T cells. IL-21 can stimulate PD-L1 expression on B cells and IL-10 induces PD-L1 on monocytes. Expression of PD-L2 is stimulated by interferons, IL-4, and GMCSF on dendritic cells *in vitro*, and the common γ chain cytokines can induce PD-L1 and to a lesser extent PD-L2 on human monocytes/macrophages [83].

PD-1 plays a role in differentiating naive T cells into Treg cells and can inhibit T-cell responses by developing Treg cells [84]. The PD-1 upregulation is a consequence of the activation of T cells, which is essential to the immune responses. PD-1 expression in Tregs is indispensable for their suppressive functions, and loss of PD1 expression accelerates the generation of Tregs which lose Foxp3 expression and produce pro-inflammatory cytokines and thereby flare autoimmunity [84]. Tregs and the PD-1/ PD-L axis are both critical to immune responses, elimination of either can result in the breakdown of tolerance and the development of autoimmunity. The PD-1/PD-L pathway can prevent autoreactive T cells and protect against autoimmunity. Treg cells induced by the PD-1 pathway can also help maintain immune homeostasis, maintaining the activation threshold for T cells to protect against autoimmunity [81, 82].

Although the PD1 pathway has received considerable attention for its roles in T cell exhaustion and tumor immunosuppression, PD1 cannot be considered a specific molecule for cell exhaustion [85]. In fact, T cells express PD1 during activation, thus being a marker for effector T cells. PD1 is expressed by subsets of tolerant T cells, regulatory T cells, follicular helper T cells, follicular regulatory T cells, and memory T cells. In addition, it is expressed on B cells, NK cells, and some myeloid cells. Expression of PD1 can be found in CD8 + T cells of healthy humans, and these cells do not resemble exhausted T cell populations [85, 86].

Polymorphisms have been described in the gene *Pdcd1* that confer susceptibility to development of autoimmune diseases in humans. Many single nucleotide polymorphisms have been reported and approximately most of them are located in the intron regions of the structural gene [87].

Some of the most studied are the PD-1.1 located in the promoter region, PD-1.2 located in intron 2, PD-1.3 and PD-1.4 located at intron 4, PD-1.9 and PD-1.5 in exon 5 and PD-1.6 at position 32 of the untranslated region. Exist different haplotypes of these SNPs in families Caucasian and it is known that PD-1.1, PD-1.2 and PD-1.9 are in linkage imbalance, while the PD-1.4 and PD-1.5 positions they form a different block [88, 89].

PD1 polymorphisms are associated with susceptibility to a variety of autoimmune conditions including systemic lupus erythematosus, rheumatoid arthritis, and progression in multiple sclerosis [89], but it is not yet clear if these SNPs are causative or simply correlative. Furthermore, autoantibodies against PDL1 have been found in patients with rheumatoid arthritis and correlate with disease activity [89].

Ponce de León et al. [90] have focused the alteration of PD-1/PD-L1 pathway in celiac disease. Levels of sPD-1 was considerably higher in the serum of patients with celiac disease compared with health controls. A negative expression of PD1 in intestinal epithelial cells and lamina propria cells of active CD patients. PD-1 protein expression in CD4 + and CD8 + T cells decreases significantly in patients with CD. In this way, PD-1 function would be compromised in CD4 + and CD8 + T cells, indicating an inappropriate activation state [90]. In CD, a deregulation of immune suppression mechanisms appears, which can lead to abnormal and persistent activation of T cells and the production of cytokines. Without PD1, excessive immune-mediated tissue damage can lead to devastating consequences, because PD1 plays crucial roles in central and peripheral T cell tolerance, aiding in the protection of

self-tissues from autoimmune responses. The co-delivery of soluble PD-1 could increase the maturation of DCs, which could accompanied by upregulation of DC maturation markers such as major histocompatibility complex II (MHC II) [90]. DC maturation is mediated by activated T lymphocytes, therefore sPD-1-regulated DC maturation may be influenced by increased T cell responses [90].

The soluble isoform is likely to have antagonistic effects on PD-1 by interfering with its signaling pathway, particularly considering that PD-1Δ3 still retains the ability to bind to PD-L1/PD-L2 receptors [90]. In patients with CD, excessive soluble PD-1 could serve as an "antibody" to block the PD-1/PD-Ls pathway and lead to aberrant T-cell proliferation. If, for example, CD8 + T-cell responses are not adequately controlled, severe immunopathology can result from the production of pro-inflammatory cytokines, such as IFN-γ and TNF-α [91].

Soluble PD-1 can promote T-cell responses through blocking the PD-1/PD-Ls pathway. IFN-γ is crucial for this process but also contributes to the upregulation of PD-L1 which indicates that sPD-1 plays a crucial role not only during the phase of T-cell exhaustion but also during primary T-cell activation and that sPD-1 can be used as an adjuvant to increase T-cell immunity [91, 92]. These findings suggest that at the time of clinical diagnosis of CD, T cells can exhibit features of immune exhaustion. It is not yet known what factor(s) contribute to the dysregulated PD-1 expression and may have increased susceptibility to the autoimmune complications of CD. The PD-1 and PD-L1 levels in the serum and intestinal biopsies of CD patients may be relevant to the determination of a possible correlation between markers of the autoimmune response, inflammation, and disease activity.

3. Future directions

The immune cell activation in the setting of immune checkpoint inhibitors results in unmasking of gluten sensitivity in genetically susceptible people, leading to expansion of previously self-reactive CD4+ T cells and subsequent CD8+ T cell-induced tissue destruction. Soluble immune checkpoint molecules constitute the emerging novel mediators in immune regulation. The relationship between celiac disease and the level of soluble immune checkpoints as sCTLA4, sHLA-G, sPD-1, and sPD-L1 has been shown.

3.1 Immune checkpoints cooperation

The immune checkpoint molecules may be implicated in biological mechanisms underlying celiac disease. Some immune checkpoint molecules serve as inhibitory signaling mediators to maintain immune tolerance, especially in the adaptive immune compartment. There are two forms of these molecules: the surface receptor or membrane-bound and cell-free soluble molecules. The membrane-bound CTLA4, HLA-G, PD-1, PD-L1 regulate T cell homeostasis, inhibit autoreactive T cells, and drive peripheral tolerance in cancer, pregnancy, and sepsis. 12,13 They also promote T regulatory cell development and inhibit the effector T cell differentiation and cytokine production leading to immunosuppression [93]. On the other hand, the soluble forms of these immune checkpoint molecules were discovered later, and their biological functions have gradually been elucidated. The immune regulatory effect of soluble PD-L1, sCTLA-4, and sHLA-G can trigger Treg differentiation and T cells apoptosis due to retention of their receptor [94].

Circulating soluble PD-1, CTLA4 and HLA-G could take part in modulating immune tolerance causing disturbances in the molecular mechanisms responsible for maintenance of balance between effector and regulatory components of the

immune system in celiac disease. HLA-G and IDO acting independently, both molecules would be complementary in inducing efficient tolerance status. HLA-G1/HLA-G5 and IDO molecules act on alloreactive T-cell proliferation through two distinct inhibitory pathways. However, as IDO expression is tightly regulated and responsive to inflammatory mediators, HLA-G may indirectly modulate IDO by up-regulating the production of such mediators. For instance, by up-regulating the expression of IL-10, HLA-G may boost the IDO pathway.

The HLA-G/ILT2/ILT4 interactions actually target a broader array of immune effectors than the B7/CTLA4 and PD-1/PD-L1 pathways, since CTLA4 and PD-1 are expressed only on T cells, whereas ILT2 and ILT4 are differentially expressed on NK, T, and B cells as well as monocytes, dendritic cells (DCs), and neutrophils and thus may inhibit the early phases of an immune response (PD-1/PD-L1), or the later phases (B7/CTLA4) [93].

3.2 Gene dysregulation in celiac disease

The detection of the spliced/soluble variant of these immune checkpoints from CD patients suggests that the soluble form of HLA-G, CTLA-4 and PD1 molecules does not result from a cleavage of the full-length form. The potential genetic associations of several polymorphisms to susceptibility to autoimmune diseases have been described, Splicing machinery would act as a biosensor to adapt gene expression to pathophysiological conditions.

Gene dysregulation of these genes could lead to an imbalance in the splice variants present in the cells at any given time. The existence of specific factors in the serum of celiac patients, such as peptides derived from gliadin, would be able to modulate the expression of relevant components of the splice and the function of the splicing machinery. Dietary intervention of gluten peptides can clearly alter the expression pattern of the splicing machinery in humans at risk for CD.

The alternative splicing process may represent a physiological mechanism for maintaining cellular homeostasis, as suggested by different studies that demonstrate that the nutrients can modulate gene expression and, in particular, the splicing of pre-mRNAs that encode regulatory proteins. Minimal disturbances in the alternative splicing process can lead to the generation of deficient proteins that contribute to several human diseases. So, the splicing process may represent an adaptive mechanism in response to different nutritional conditions, and that this mechanism could be in place not only in circulating PBMCs but may also operate in cell types from other tissues and organs tightly coupled to nutrient-dependent metabolic homeostasis, e.g., intestine. So, specifically gluten can modulate processes required for cell homeostasis through the alteration of gene expression and, particularly, the splicing of pre-mRNAs encoding key regulatory proteins.

4. Conclusions

Further investigation on the determination of immunological interactions and biological functions by immune checkpoints in celiac disease is needed to deepen our understanding of the underlying disease mechanism in ourquest for diagnostic and therapeutic target in celiac disease.

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Author details

Isabel Torres[1*], Miguel Ángel López Casado[2], Teresa Palomeque[1] and Pedro Lorite[1]

1 Department of Experimental Biology, Campus Universitario Las Lagunillas, Jaén, Spain

2 Departamento de Gastroenterología Pediátrica, Hospital Virgen de las Nieves, Granada, Spain

*Address all correspondence to: mitorres@ujaen.es

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