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HLA in Gastrointestinal Inflammatory Disorders

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1. Introduction

1.1. HLA GENE

The human leukocyte antigen (HLA) region is located on chromosome 6p21.3 and encodes genes for the major histocompatibility complex (MHC). MHC genes are among the most polymorphic genes in the vertebrate genome. HLA region contains hundreds of genes with immunological function that is characterized by a high gene density, variability and extensive linkage disequilibrium [1]. HLA encodes proteins with critical role in immunity, including antigen processing and presentation, and self-recognition by immune cells, as ligands receptors, cytokines, signaling factors, heat shock proteins, and transcription regulators. The HLA region is involved in many biological processes such as histocompatibility, inflammation, ligands for immune cell receptors, and the complement cascade [2].

HLA genes have many possible variations, allowing each person's immune system to react to a wide range of foreign invaders. Some HLA genes have hundreds of identified versions (alleles); more than 100 diseases involving abnormal immune function and some forms of cancer have been associated with different alleles of HLA genes [3,4]. Is paradoxical that genes evolved to protect the host against infectious diseases are cause of many genetic disorders themselves. It is often unclear what role HLA genes play in the risk of developing these diseases.

The MHC is the most gene-dense region of the mammalian genome playing a key role in immune defense and autoimmunity [5]. The complex is divided into three subgroups (MHC classes I, II, and III). The class I region is located at the telomeric end of the MHC and encodes heterodimeric peptide-binding proteins as well as antigen processing molecule. The class I family is divided into classical (HLA-A, -B, -C) and non-classical (HLA-E, -F and -G) genes. The classical genes are highly polymorphic and constitutively expressed by most somatic cells, and are co-dominantly expressed on the cell surface and responsible for presenting intracell-

ularly derived peptides to CD8-positive T cells. On the cell surface, these proteins are bound to protein fragments (peptides) that have been exported from within the cell. MHC class I proteins display these peptides to the immune system.

The human MHC class I chain-related genes (MICA and MICB) are located within the HLA class I region of chromosome 6. MICA/MICB organization, expression and products differ considerably from classical HLA class I genes. [6] Mapping studies identified seven MIC loci (MICA–MICG), of which only MICA and MICB encode transcripts, while MICC, MICD, MICE, MICE, and MICG are pseudogenes. MICA gene is located 47 kb centromeric to HLA-B [7] in the MHC class I region and MICB gene is located near to MICA, shares identical structures, functions, and patterns of expression. These genes are highly polymorphic, with several MICA and MICB alleles recognized [8]. A high degree of linkage disequilibrium exists between MICA, MICB, and HLA-B. MICA and MICB gene polymorphisms have been found associated with autoimmune diseases [9] including insulin-dependent diabetes mellitus [10], Addison's disease [11], celiac disease [12, 13], rheumatoid arthritis [14, 15], Behcet's disease [16], and IBD [17–19]

These molecules do not bind β 2 microglobulin or peptide typical of HLA class I. The highly polymorphic MICA and MICB encode stress-inducible glycoproteins expressed on a variety of epithelial cells including intestinal epithelial cells. Interaction with the receptor NKG2D is likely to provide an important costimulatory signal for the activation of natural killer (NK) cells, macrophages, CD8+ $\alpha\beta$, and $\gamma\delta$ T cells [20]. MICA/MICB encodes proteins that interact with different T-cell receptors in response to stress as infection, as heat-shock, oxidative stress or neoplastic transformation. Gamma/delta T cells are concentrated in the intestinal mucosa and appear to have a prominent role in recognizing small bacterial phosphoantigens and other antigens presented by MICA/MICB proteins. Gamma/delta T cells have potent cytotoxic activity and have been considered a link between innate and adaptative immunity [20].

MHC class II encodes heterodimeric peptide-binding proteins, and proteins that modulate peptide loading onto MHC class II proteins in the lysosomal compartment. Among the most studied MHC genes are the four classes of human leukocyte antigen (HLA) genes that encode cell-surface antigen-presenting proteins. The class II region lies at the centromeric end of the MHC and encodes HLA class genes HLA-DRA, -DRB1, -DRB3, -DRB4, -DRB5, -DQA1, -DQB1, -DPA1 and -DPB1. HLA class II expression is limited to cells involved in immune responses, where these molecules present extracellularly derived peptides to CD4-positive T cells.

In HLA-DR, the polymorphic variation is provided by the β -chain alone as the α -chain is monomorphic. However, in DQ and DP, both the α -chains and the β -chains are polymorphic. As a result, unique DQ and DP molecules can be formed with α - and β -chains encoded on the same chromosome (*i.e.* encoded in *cis*) or on opposite chromosomes (*i.e.* encoded in *trans*) [21]. The occurrence of *trans*-encoded HLA class II molecules is well documented in the literature [22]. However, evidence suggests that not every α - and β -chain pairing will form a stable heterodimer [23, 24]. It is generally considered that alleles of DQ- α - and DQ- β -chains pair up predominantly in *cis* rather than in *trans* [23, 25].

Located between the class I and class II regions lies the class III region where a number of non-HLA genes with immune function are located. These genes are located on the 1100 kb section between class I and class II genes inside the MHC, and contain about 70 genes. The complement gene block is inherited as a genetic unit known as complotype. Each complotype codifies for the synthesis of complement classic pathway C2, C4A, C4b factors, and alternative pathway B factor, which may suggest that alterations within the region might affect the host's defense system and introduce a complement deficiency [26].

HLA-G is a non-classical MHC class I molecule displaying restricted tissue expression and low polymorphism. Under normal conditions expression of the HLA-G protein is restricted to the feto-maternal interface on the extravillous cytotrophoblast protecting the fetal semi-allograft against the maternal immune system and to the thymus in adults, creating a general state of tolerance. HLA-G exhibit tolerogenic properties via interaction with inhibitory receptors presented in natural killer (NK) cells, T cells and antigen-presenting cells (APC) [27].

HLA-G expression is up-regulated under pathological conditions in inflammatory diseases such as psoriasis [28] and atopic dermatitis [29], IBD [30], celiac disease [31], in viral infection [32], and organ transplants [34]. The main function of HLA-G is suppression of several immune processes carrying out inhibitory effects on cytotoxicity by NK cells and CTL, T cell proliferative responses and maturation of dendritic cells. These inhibitory effects are mediated by ligation of HLA-G and inhibitory receptors such as ITL2, ITL4 and KIR2DL4 on the surface of immunocompetent cells [34].

HLA-G exhibits low allelic polymorphism in comparison with the classical MHC class I molecules. HLA-G alleles are known at the nucleotide level resulting in seven different proteins [35, 36]. Seven protein isoforms, four membrane bound (HLA-G1, -G2, -G3 and -G4) and three soluble (HLA-G5, -G6 and -G7), are generated by alternative splicing [37, 38]. Further nucleotide polymorphisms have been described within the non-coding region of the HLA-G gene, 18 single-nucleotide polymorphisms (SNPs) in the promoter region and a 14-bp deletion polymorphism in exon 8 (rs16375) encoding for the 3'untranslated region [39,40]. The latter polymorphism is potentially functional influencing transcript levels and splicing [41]. In addition to the allelic polymorphism the HLA-G gene shows a deletion/insertion polymorphism of a 14 base pairs sequence (14bp) in the exon 8 at the 3 untranslated region.

2. Inflammatory bowel disease (IBD)

2.1. HLA class I and class II

Inflammatory bowel diseases (IBDs) are complex, multifactorial disorders that comprise Crohn's disease (CD) and ulcerative colitis (UC). Genome-wide association (GWA) studies have identified approximately 100 loci that are significantly associated with IBD [42-44]. These loci implicate a diverse array of genes and pathophysiologic mechanisms, including microbe recognition, lymphocyte activation, cytokine signaling, and intestinal epithelial defense. Although CD and UC are both associated with genomic regions that implicate products of

genes involved in leukocyte trafficking, there is evidence for association patterns that are distinct between CD and UC [45].

Evidence from family and twin studies suggests that genetics plays an important role in predisposing an individual to develop ulcerative colitis and Crohn's disease. Further evidence of a genetic predisposition comes from studies of the association between the human leukocyte antigen (HLA) system and IBD [46]. The immunogenetic predisposition may be considered an important requirement for the development of IBD, as several markers of human major histocompatibility complex. HLA complex on chromosome 6 is the most extensively studied genetic region in inflammatory bowel disease [46]. Although it is difficult to estimate the importance of this region in determining overall genetic susceptibility, calculations derived from studies of HLA allele sharing within families suggest that this region contributes between 10%-33% of the total genetic risk of crohn's disease[47] and 64%-100% of the total genetic risk of ulcerative colitis[48].

Antigen presentation by intestinal epithelial cells (IEC) is crucial for intestinal homeostasis [49]. Results from Bisping et al. [50] showed an activation of CD8+effector T cells during active IBD, a process related to MHC I. These data emphasize the importance of the MHC I and II-associated antigen presentation by IEC for the homeostasis of the gut. Disturbances MHC I and II-related presentation pathways in IEC appear to be involved in an altered activation of CD4(+) and CD8(+) T cells in inflammatory bowel disease [51]

The mechanism by which classical HLA class II genes exert their influence in IBD is unclear, although a number of hypotheses have been postulated. Polymorphism in these molecules is concentrated around specific pockets of the binding groove that interact with critical side-chains or 'anchor' residues of a peptide. Thus different HLA molecules may bind preferentially to different peptides, or bind the same peptide with varying affinity. In IBD, cross reactivity (known as "molecular mimicry") may exist between the peptides derived from bacterial luminal flora and from self- antigens present in the gut. This may lead to the generation of auto reactive T cells which contribute to disease pathogenesis through either stimulation or inhibition of the immune system [46].

The recent developments in IBD research point clearly to a defect in the mucosal barrier of the gut as the pivotal and primary pathogenic mechanism [52]. Subsequently, mucosal tolerance is disturbed and effector T cells are stimulated constantly, perpetuating the inflammatory process. In CD, inflammation is driven by CD4+ T helper type 1 (Th1) and Th17 cells that, among others, secrete IFN- γ , TNF- α , IL-17, IL-21, IL-22 and IL-26. The immune response in UC appears to be less polarized, but reveals a strong Th2 component with IL-4, IL-5 and IL-13. The role of CD8+ effector T cells is still uncertain. Activated DC is considered to play the major role in antigen presentation and activation of the afore-mentioned effector T cells [49].

HLA genes are the most extensively studied genetic regions in ulcerative colitis (UC). There is consistent evidence that supports the variable incidence of the disease among ethnic groups, familial aggregation, monozygotic twins and the increased frequency of UC in certain genetic syndromes. There are several HLA alleles associated with different clinical features in UC patients, but they may change according to the ethnic group, such as HLADRB52 and HLA-

DRB1*02 in Japanese [53], HLADRB1*03 in Caucasians [26, 46, 53–56,], the HLA-B35 in Jews [56], HLA-A19, HLA-A33 in Asians [26, 57] and HLADRB1*04 in the Amerindian population in Mexico [58].

The HLA-DRB1 allele is the most studied in inflammatory bowel disease (IBD). There are existing data that some of these alleles may confer risk as well as protective characteristics. There is a consistent association between HLA-DRB1*0103 and severe UC in American and European populations. It is one of the most remarkable allelic associations that provides evidence about the association between HLA-DRB1*0103 and extensive colitis, a severe course of disease, extra-intestinal manifestations (EIMs) and disease activity [59]. A number of HLA associations have been described with the extra-intestinal manifestations of IBD. It is known the association between DRB1*0103 and the extra-intestinal manifestation in patients with colonic Crohn's disease. In this sense, symmetrical arthritis is associated with HLA-B*44 [60]. Uveitis has also been associated with DRB1*0103 and HLA-B*27, and erythema nodosum with the TNF promoter SNP TNF-1031C [61].

Okada Y et al. [62] have demonstrated that a particular HLA haplotype, HLA-Cw*1202-B*5201-DRB1*1502, independently confers a susceptible effect on UC, but has a protective effect on CD in Japanese population. This study showed that one haplotype extending throughout the MHC class I, III, and II regions confers opposite directions of effects on UC and CD. This haplotype accounted for two thirds of the difference of the genetic risks between UC and CD in the MHC region, suggesting its substantial role in the etiology of IBD. The specific pathogens recognized by HLA-Cw*1202-B*5201-DRB1*1502 haplotype will promote the inappropriate proliferation and differentiation of naïve CD4₊ T cells and induce the Th1/Th2/Treg imbalance in the intestinal immune response. This imbalance will contribute to the opposite directions of the susceptibility to UC and CD. Contrary to these results, the comparative study for IBD in European populations did not demonstrate the distinct effects in the MHC region. One probable explanation for this discrepancy would be the ethnic differences of haplotype frequencies [62, 63].

Among HLA class I genes, *B52* conferred the greatest risk for UC, whereas *Cw8* and *B21* conferred the greatest risk for CD [64]. GWAS studies have highlighted the importance of the HLA region in IBD, with greater association evidence of HLA variations to risk with UC than CD. A recent meta-analysis of nearly 7000 cases of UC and 20,000 controls reported the strongest association with SNP rs9268853, near HLA DR9.9. In contrast, a meta-analysis of 6300 cases of CD and 15000 controls found a relatively modest level of association for CD within the HLA region, strongest with SNP rs1799964; 21 loci outside the HLA region had more robust significance [65].

Clinical importance of differential diagnosis of UC and CD has been recognized, and incorporation of genetic markers in the diagnosis is proposed as a promising clue [66, 67]. The identified HLA haplotype distinguishes UC and CD, which would have more impacts than the previously evaluated variants [67]. Thus, utilization of the genotype information of the HLA haplotype, or alternatively the SNP(s) in LD with it, might contribute to improvements of diagnostic approaches on UC and CD.

2.2. Non classical I genes

Intestinal epithelia cells express several non-classical MHC I molecules and are regulated by distinct signals, supporting the hypothesis that these may be involved in local immunoregulation in the intestine [68]. The expression of non-classical MHC I molecules is altered in IBD (all absent in ulcerative colitis [UC] and selective absence of CD1d in Crohn's disease [CD]), providing further evidence for the scenario that non-classical MHC I molecules are involved in T-cell regulation. Distinct regulatory T-cell populations may be regulated by different non-classical class I molecules and that there may be differential regulation of these molecules on intestinal epithelial cells

2.3. MICA/MICB

Some not classical genes related to the class I genes such as MHC class I chain-related gene A (MICA) and MHC class I-related chain B (MICB), are expressed in the basolateral cells in the gastric epithelium, fibroblasts, endothelial and dendritic cells. It is known that its expression rises during viral and bacterial infections [69]. Some genetic studies in patients with IBD have found associations with MICA-A6 and HLA-B52 in Japanese patients with UC [70], MICA*010 and HLA-B*1501 in English patients with fistulous CD [71]. MICA and MICB bind to an activating receptor natural killer group 2D (NKG2D) which is expressed on NK cells, T cells and macrophages and the interactions between these receptors may directly stimulate cell cytotoxicity as well as providing co-stimulation for NK and T cell activation. Several MICA alleles have been shown to alter the binding affinity with NKG2D suggesting they may exert a functional effect on immune activation [26]

Several works showed differences in ethnic and regional living environment that would influence on the MICA/MICB gene distribution. Japanese UC patients showed an increased frequency of allele A6 of the MICA exon 5 trinucleotide microsatellite polymorphism as compared with unaffected controls [72], although a follow up study by this group suggested that the significant increase previously seen was attributable to linkage disequilibrium with HLA-B52 [73]. While Orchard et al. [74] related the MICA × 007 with susceptibility to UC in a British population, Glas et al. [75] failed to show a similar association in their German cohort.

López-Hernández et al. [76] have studied allele polymorphism and the functionally relevant dimorphism (129val/met) of MICA gene in IBD patients in Spanish population. The presence of MICA-129 met/met and MICA-129 val/met genotypes may modify NK, $\gamma\delta$, and T CD8 lymphocytes activation, and thereby may allow an exacerbated immune response in intestinal environment with a strong inflammatory component. This study showed that MICA-129 val/met genotype was less common in IBD patients than in controls, suggesting that it could be also associated with protection against the disease in these patients. Also, The MICA-129 gene polymorphism was associated with UC in Chinese patients, and the soluble MICA levels in UC patients were higher than those in healthy controls. Based on the role of the *MICA-129* gene in NKG2D-receptor activation, these findings might indicate that the host innate immune response is associated with UC [77].

In recent studies on MIC genes susceptible to UC, MICA*007 [78], MICA*00801, and MICA-A6 [79] were shown to be associated with UC onset in Japan. MICA-A5.1/A5.1 homozygous genotype MICA-A5.1 [80] and MICB-CA18 [81] alleles were associated with UC in the Chinese Han population. Li et al. [82] showed that the frequency of MICB0106 allele was significantly higher in the UC group in a limited population in central China, especially in patients over the age of 40 years with extensive colitis, moderate and severe disease, and in those with extra-intestinal manifestations. The comparison of MICB alleles in the Chinese Han population with those in England and Spain populations showed significant differences in the distribution of the exon2–4 of MICB alleles. Also, significant differences in the distribution of the intron 1 of MICB alleles were also observed between the Chinese and other populations [82].

2.4. HLA-G

A differential pattern of HLA-G expression in CD and UC has been shown. By immunohistochemistry, increased HLA-G surface expression has been only detected in UC, whereas HLA-G expression was absent in CD and also in healthy controls [83]. HLA-G was highly expressed in intestinal tissues of UC, regardless of the intestinal location (ascending, transverse, descending colon, or ileum), and of the medical history and regimen of the patients. The expression of HLA-G was restricted to the apical surface of intestinal epithelial cells (IECs) in the epithelial layer in the intestinal mucosa and Lieberkun crypts. IECs showed intense apical staining and no immunoreactivity was found in the other mucosal cell types such as lymphocytes, macrophages or endothelial cells [83].

Torres et al. [83] suggest a role of HLA-G in the immunopathogenesis of IBD, and proposed that the analysis of HLA-G expression possibly can be used for diagnostic purposes to distinguish between CD and UC in cases of indeterminate colitis. In this sense, the study of Rizzo et al. [84] also has documented a clear difference in the production of soluble HLA-G molecules in IBD patients, confirming the presence of a different etiology and immune response mechanisms in UC and CD. HLA-G expression in UC might reflect a down-regulation of the immune response against inflammation. HLA-G expression is likely to be insufficient to protect the intestine from the inflammation after aggression is stopped. HLA-G potentially functions as a shield against inflammatory aggression. It might contribute to tissue protection by inhibiting NK cell activity and tissue infiltration by T cells and monocytes shifting the balance between T_H1 and T_H2 cells toward the T_H2 pathway. A potential role of HLA-G in the pathogenesis of UC is in line with the T_H2 pathway rather characteristic for UC. In UC, the increased expression of HLA-G might reduce clearing of intestinal micro-organisms and therefore promote a secondary chronic inflammation [83].

This differential expression pattern of HLA-G is possibly influenced by genetic variations within the HLA-G gene. The *HLA-G* gene is located in IBD3, a linkage region for inflammatory bowel disease (IBD). A 14-bp deletion polymorphism (Del+/Del-) within exon 8 of the *HLA-G* gene might influence transcription activity and is therefore of potential functional relevance.

Glas et al. [85] have found that the 14-bp deletion polymorphism in the *HLA-G* gene displays significant differences between ulcerative colitis and Crohn's disease and is associated with ileocecal resection in Crohn's disease. The allele, genotype and phenotype frequencies of the 14-bp deletion polymorphism in UC or CD displayed no significant differences when

compared with a healthy controls [85]. The allele frequencies in the control group found herein were similar to those detected in the control groups of other studies [87-89]. When the UC and CD groups were compared among each other, the Del+ phenotype and the heterozygous Del +/_ genotype were significantly increased in UC, whereas the frequency of the homozygous genotype Del/_/_ was significantly lower in UC than in CD.

The CD patients were stratified for disease behavior, for disease location/extent and for the need of ileocecal resection [85]. In CD patients with ileocecal resection, the frequency of the Del+/Del+ genotype was 47.0% compared with 27.3% in patients without ileocecal resection, whereas the two other genotypes were decreased, showing a significant association of the 14-bp deletion polymorphism. The frequency of the Del+ allele was significantly increased in CD patients with in relation to those patients without ileocecal resection (69.7 versus 52.7%), but not in the comparison of healthy controls. In this sense, HLA-G may play a role in modulating the course of CD rather than determining overall susceptibility. The differences of the 14-bp deletion HLA-G polymorphism between UC and CD found gives evidence for a contribution of the HLA-G gene in the pathogenesis of IBD. The findings of Glas et al. [85] are consistent with the differential expression pattern of HLA-G in UC compared with CD as shown by Torres et al. [83], and in agreement with the work of O'Brien et al. [89], where decreased HLA-G expression was observed in placenta samples of cases with homozygous Del - genotype.

2.5. MHC class III genes

The proteins produced from MHC class III genes exhibiting functions in immunological processes such as the cytokines TNF- α , TNF- β and the heat shock proteins [26]. The functions of some MHC genes are unknown. This raises attention when TNF- α is thought to play an important role in the pathogenesis of IBD, acting as a potent pro-inflammatory cytokine with elevated serum and tissue levels in patients with IBD [59, 90-91], and evidence show that there are specific genetic polymorphisms involving TNF- α that influence the amount of cytokine produced.

Bouma et al. [92] and Louis et al. [93] studied the allelic frequency of TNF- α gene polymorphisms at -308 position finding that polymorphism in allele 2 was decreased in UC patients as compared to normal controls. In a Mexican population with UC, the presence of TNF*2 allele was associated with the presence of this disease as compared with healthy subjects [94]. In Mexican patients with UC, an association was found between complotype SC30 (Bf*S-C2*C-C4A*3-C4B*0) and UC [95], which might suggest that activation of complement system could interfere with the disease pathogenesis.

3. Celiac disease

3.1. HLA class II

HLA is a master piece in the pathogenesis of celiac disease, as first evidenced by the strong genetic association existent between celiac disease susceptibility and certain HLA alleles [96,

97]. Celiac disease is a chronic gluten intolerance that occurs in genetically predisposed individuals. Is a chronic inflammatory disease which is a T cell-mediated inflammatory disorder with autoimmune features and it has environmental and immunologic components. It is characterized by an immune response to ingested wheat gluten and related proteins of rye and barley that leads to inflammation, villous atrophy crypt hyperplasia and lymphocyte infiltration, leading to nutrient malabsorption. A wide spectrum of clinical phenotypes is present, ranging from classical gastrointestinal manifestations to only atypical signs [96].

The prevalence of celiac disease is estimated at about 1:100 in Caucasian population but many cases remain undiagnosed, especially among adult individuals, because of the wide variability of symptoms [98, 99]. As for other autoimmune diseases, celiac disease occurs more often in female than in male subjects with a gender ratio of about 2:1 [98,100,101]. Furthermore, gluten intolerance is more frequent in at-risk groups, such as first-degree relatives of patients as well as individuals with specific genetic syndromes (Down, Turner, Williams) or autoimmune diseases (mainly type 1 diabetes, thyroiditis and multiple sclerosis) [102,103]. Celiac disease has a multifactorial inheritance, so it does not depend on specific mutations of a single gene but it is caused by a combination of environmental factors and variations in multiple genes [104,105]. Familial aggregation (10-12%) and higher concordance rates of celiac disease in monozygotic than in dizygotic twins (83-86% vs. 11%) have been confirmed, indicating that a strong genetic contribution is involved in the disease occurrence [106, 107]. Furthermore, the association with other autoimmune conditions in the same individual or in different members of the same family suggests the existence of common predisposing genes to autoimmunity [108].

The HLA is the most important genetic factor in celiac disease, and carriage of certain HLA alleles is a necessary, but not sufficient, factor for disease development HLA influence on celiac disease susceptibility showing a dose effect that implies the existence of different celiac disease risk categories attending to their HLA constitution [109]. This pathology presents a strong genetic susceptibility: approximately 90 % of celiac patients carry the HLA-DQ2 heterodimer (encoded by DQA1 * 05 and DQB1 * 02 alleles), whereas a smaller percent of subjects carry the HLA-DQ8 heterodimer (encoded by DQA1* 03 and DQB1* 0302 alleles) [110]. The HLA-DQ2 coding alleles can be encoded in *cis* on the DR3-DQ2 haplotype or in *trans* on the DR5-DQ7 / DR7-DQ2 heterozygotes [110,111], whereas the HLA-DQ8 heterodimer is encoded in *cis* on the DR4-DQ8 haplotype. DQ alleles, however, account only for 40 – 50 % of the genetic contribution for CD [112]. It has been reported that not all the HLA DR3 / DQ2 haplotypes confer equal susceptibility to CD, suggesting that DQ2 is not the only HLA-linked genetic risk factor [113].

Individuals can be classified in high or intermediate CD risk according to the number of DQA1*05- and DQB1*02-carrying alleles. Homozygosity for DQ2.5 *cis* and heterozygosity for DQ2.5 *cis* with a chromosome possessing a second DQB1*02allele (DQ2.2) confer the highest risk to develop CD. Heterozygosity or DQ2.5 *cis* in individuals with a single copy of DQB1*02 (non-DQ2.2) or presence of DQ2.5 *trans* confer intermediate risk [109]. The influence of HLADQ8 (genetically DQA1*03, DQB1*03:02) on the disease is already known. This molecule is present in almost all the celiac patients without DQ2.5. However, the genetic influence of

the HLA region in CD is not limited to the factors coding DQ2 or DQ8, and several works have attempted to discover new susceptibility factors [109].

In celiac disease, T cell stimulation due to gluten-derived peptides depends on the number and type of HLA-DQ2 molecules expressed. DQ2.5 molecules can bind a high repertoire of gluten peptides, but only a restricted subset is bound to DQ2.2 molecules, which reduce the immunogenicity of DQ2.2. Additionally, the number of these DQ molecules is also a relevant factor in T cell stimulation and this depends on the number of specific alleles in DQA1 and DQB1 loci, which determines the possible $\alpha\beta$ -chain combinations constituting the DQ heterodimers [114].

The HLA dose effect is also influenced by differences in the kinetic stability of the interaction between HLA molecules and gluten derived peptides, key factor for development of T cell responses against gluten [115,116]. For most peptide ligands, DQ2.5 shows higher binding stability than DQ2.2. The high kinetic stability of peptide-MHC is a key factor for establishment of antigluten T-cell responses and the development of celiac disease. Kinetic stability of peptide-MHC complexes has been shown to be decisive for antigen-presenting cells to successfully activate naïve T cells in the lymph node [117]. Polymorphism in DQ2.2 results in a lower kinetic binding stability of commonly recognized DQ2.5-restricted T-cell epitopes when tested for binding to DQ2.2. The authors suggest that this phenomenon might explain the large difference in risk of celiac disease for these homologous DQ molecules [118]. Moreover, gluten epitopes recognized by DQ2.2 patients would be peptides that form stable peptide-MHC complexes and T cells reactive with known DQ2.5-restricted gluten epitopes with fast off-rate would be rarely found [116].

Bodd et al. [119] have reported the presence of DQ9-restricted gluten reactive T cells recognizing the DQ8-glut-1 epitope, an epitope previously described to be recognized by DQ8 patients, in the small intestine of a celiac patient expressing DQ9. This epitope appears to be the dominant DQ9-restricted epitope in this patient and binds particularly well to DQ9 compared with other DQ8 gluten epitopes. These authors found that DQ9-restricted gluten-reactive CD4⁺ T cells could be isolated from the small intestine of a CD patient expressing DQ9 and DQ2.2.

HLA typing does not have an absolute diagnostic value but allows assess the CD relative risk; a positive test is indicative of genetic susceptibility but does not necessarily mean the disease development [97]. A negative test has a more significant value because gluten intolerance rarely occurs in the absence of specific HLA predisposing alleles. HLA genes are stable markers throughout life, so their typing can discriminate genetically CD-susceptible or not susceptible individuals before any clinical or serological signs. HLA test is increasingly considered as a solid support in the diagnostic algorithm of CD. New European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) guidelines for the diagnosis of CD have established that duodenal biopsy can be omitted in cases with elevated serum anti-TG2 antibodies positive and at-risk HLA [120].

3.2. MICA/MICB

MICA and MICB are interesting candidates as susceptibility genes in celiac disease. It has been demonstrated that peptides from gliadin could induce the expression of MICA in gut epithelium of celiac patients [121]. Moreover, the participation of the MICA/NKG2D pathway in the destruction of intestinal epithelium by intraepithelial T lymphocytes in celiac disease has been revealed [122].

Aberrant MICA responses have been implicated in celiac disease. MICA/B expression was reduced in duodenal samples from patients under a gluten-free diet, reflecting a possible link between the ongoing inflammatory process induced by gluten ingestion and MICA/B expression. Therefore, considering the pattern of MICA/B expression in different cell lineages observed, signals for induction of MICA/B may be part of a more general mechanism associated to the ongoing inflammatory process in the small intestine in untreated celiac patients. Several studies on intestinal tissue, isolated cells from intestinal mucosa or epithelial cell lines support a link between cellular (heat, oxidative and ER) stress and mucosal damage. In their study, the authors also observed expression of MICA/MICB in B and T lymphocytes [123,124]. MICA expression in activated T lymphocytes confers susceptibility to NK cell-mediated cytotoxicity [125]. Cell surface MICA/B expression may act to negatively regulate T cell function by decreasing of IFN- γ production and cytotoxicity and reduce tissue damage by regulatory mechanisms via NK/T cell interaction.

High production of IL-15 in intestinal mucosa in active celiac disease has been shown to trigger enterocyte apoptosis via the induction of cell surface MICA, which in turn interacts with the activating NKG2D receptor present in intestinal epithelial lymphocytes. Cytotoxic activity of IELs is also potentiated by IL-15 through activation of JNK and ERK pathways [126-128]. Though MICA/B confers susceptibility to NKG2D-mediated killing of enterocytes by intraepithelial NK and CD8⁺ T cells in untreated celiac disease, these results suggest that MICA/B expression may also regulate cell survival of other cells in the intestinal mucosa.

Allegretti et al [129] observed a more ubiquitous distribution of MICA/B expression. In enterocytes, the expression was mainly found in the cytoplasm as peri- and/or supra-nuclear aggregates. The analysis of the intraepithelial compartment, which contains different lymphocytes, most of them CD7⁺ cells, revealed the expression of MICA/B in lymphocytes in celiacs and control samples. The authors found coarse MICA/B aggregates in the cytoplasm of CD7⁺ cells; which were more frequently observed in mild enteropathy samples. The intracellular location of MICA in intraepithelial and lamina propria T cells may hinder their recognition by NKG2D-expressing cells avoiding the control of over activated T cells [129]. The results of these authors suggest that expression of MICA/B in the intestinal mucosa of celiac patients is linked to deregulation of mucosa homeostasis in which the stress response plays an active role. MICA/B may play a more general role than previously thought in gut immunobiology.

Rodríguez- Rodero et al. [130] have found that the MICB promoter is polymorphic and some of them were seen to be associated with celiac disease. The variants 45944-- , 46219G, and 46286G, as parts of the MICB*008 and MICB*002 promoters (Haplotype 3), were found to be significantly overrepresented in celiac patients. In addition to the MICB*008 allele, the

MICB*002 allele is included in the extended haplotypes EH62.1, EH60.1, EH51.1, and EH18.2 [131], which are frequently found in Caucasian populations. These haplotypes carry one or two susceptibility chains (DQA1*0501, DQB1*0201, DQB1*0202, DQA1*03, and DQB1*0302). MICA-A5.1 transmembrane polymorphism (MICA*00801) is associated with a risk of atypical CD and that this was found to be independent of the known susceptibility extended haplotype EH8.1 [132]. A double dose of the MICA 5.1 allele could also predispose to the onset of gastrointestinal symptoms-celiac disease [133].

3.3. HLA-G

Celiac disease is always associated with the HLADQ heterodimer encoded by DQA1*0501 and DQB1*0201 alleles, although this gene do not explain the entire genetic susceptibility to gluten intolerance [96, 97]. Therefore, it has been suggested that other genes might predispose to celiac disease. HLA-G is a molecule of immune tolerance implicated in several inflammatory diseases. Consequently, it is interesting to study the effect of this molecule in the development of celiac disease.

Torres et al. [134] have demonstrated an association of celiac disease with HLA-G expression. They have described the expression of soluble HLA-G in biopsy samples and in serum from patients with celiac disease. Conversely, membrane HLA-G molecules were not expressed in celiac patients. The lack of membrane HLA-G expression may be linked to a specific regulatory process in the alternative splicing of the primary HLA-G transcript, which could favour selection of the soluble isoforms. The enhancer expression of soluble HLA-G in celiac disease could be due as part of a mechanism to try restore the tolerance process towards oral antigens in a disease caused by loss of tolerance to dietary antigens. A powerful anti-inflammatory response to gliadin might occur during the development of the disease with uncontrolled production of HLA-G that counteract the inflammation or/and may cause recruitment of intra-epithelial lymphocytes, maintaining the intestinal lesions [31].

A number of functional HLA-G gene polymorphisms have been identified, including a 14 bp deletion/ insertion polymorphism (located at the 3' UTR of the gene, in exon 8; rs1704) associated with differences in the pattern of alternatively spliced mRNA isoforms and in concentration of sHLA-G; moreover, the inserted allele affects the mRNA stability [134, 135]. Fabriset et al. [136] have analyzed the 14 bp deletion / insertion polymorphism in a group of celiac patients and healthy individuals, stratified for the presence of HLA-DQ2 genotype, to evaluate the possible association of the HLA-G 14 bp deletion/ insertion polymorphism with the disease. These authors found significant differences in the frequencies of both the 14 bp inserted (I) / deleted (d) alleles and genotypes when comparing celiac patients with healthy controls. The higher frequency of the I allele in celiac patients as compared to healthy controls allowed to hypothesize that HLA-G molecules are involved in the susceptibility to celiac disease, as the presence of the inserted allele associated with an increased susceptibility to this pathology. The presence of the I allele confers an increased risk of celiac disease in addition to the risk conferred by HLADQ2 alone and that subjects that carry both DQ2 and HLA-G I alleles have an increased risk of celiac disease than subjects that carry DQ2 but not the I allele [136].

The 14 bp inserted allele can affect the HLA-G synthesis that is induced in celiac disease, by influencing the pattern of mRNA HLA-G splicing and by affecting the mRNA stability [135, 136]. In healthy individuals the intestinal immunity is set toward tolerance to ingested antigens, and high concentrations of anti-inflammatory cytokines are normally found in the intestine. Celiac disease is instead associated with production of pro-inflammatory cytokines such as IFN- γ and IL-15. In addition, HLA-G is induced by IFN- γ in mononuclear cells, indicating that HLA-G might constitute a pathway to protect tissues from the infiltration of T cells [137].

4. Conclusions

Today, the HLA complex occupies a central position in basic and clinical immunology. This chapter reviews current knowledge of the role of HLA complex genes in IBD and celiac disease susceptibility and phenotype, and shows the factors currently limiting the translation of this knowledge to clinical practice. Interest in the HLA complex in IBD and celiac disease has traditionally focused on association with the classical class II HLA alleles, but recent insights into the biological function of other genes encoded within this region have led investigators to a more diverse exploration of this region. The well characterization of these genes potentially will lead to the identification of therapeutic agents and clinical assessment of phenotype and prognosis in patients with these intestinal disorders. The HLA region represents the genome's highest concentration of potential biomarkers for most studied diseases. Specific HLA genotypes have already been associated with sensitivities to five marketed drugs and are recurrently being investigated as biomarkers in several clinical trials [138].

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