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The role of Haptoglobin and its related protein, Zonulin, in inflammatory bowel disease

Tim Vanuytsel, Séverine Vermeire and Isabelle Cleynen*

Department of Clinical and Experimental Medicine; TARGID; KU Leuven

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Crohn's disease (CD) and ulcerative colitis (UC), collectively called inflammatory bowel disease (IBD), are immune-mediated conditions characterized by a chronic inflammation of the gut. Their precise etiology is unknown, although an increased intestinal permeability has been shown to play a central role in the pathogenesis of IBD. The intestinal epithelium provides the largest interface between the external environment and the host, and is thus a crucial regulation site of innate and adaptive immunity. Zonulin is one of the few known physiological mediators of paracellular intestinal permeability. It was found upregulated in different immune diseases like Celiac disease and Type 1 Diabetes (T1D). Recently, human zonulin was identified as prehaptoglobin-2 (pre-HP2) which before only had been regarded as the inactive precursor for HP2. Haptoglobin (HP) is a hemoglobin-binding protein with immunomodulatory properties. Its gene harbors a common polymorphism with 2 different alleles: *HP1* and *HP2*. Allele *HP2* and genotype *HP22* has been shown to be overrepresented in different immune diseases like Rheumatoid Arthritis (RA), Systemic Lupus Erythematosus (SLE) and T1D, and has also been found to be more frequent in patients with IBD (UC and CD) than in healthy controls.

In order to get some clues about the mechanism of action of HP(2) in IBD pathogenesis, we here review the current state of knowledge about zonulin and haptoglobin structure and function, and their plausible role in immune mediated diseases with an emphasis on IBD.

Introduction: Role of Barrier Integrity/Intestinal Permeability in IBD Etiology

The single cell layer of the intestinal epithelium forms the largest barrier between the external environment and the host. The intestinal mucosa is in continuous contact with microorganisms and dietary antigens, creating a crucial site for regulation of both innate and adaptive immunity.[1] Since the plasma membrane is virtually impermeable to hydrophilic solutes in the absence of specific transporters, passive flux of small water-soluble solutes and ions occurs mainly via the paracellular route, which is controlled by the apical junctional complex.[2,3] Tight junctions, the principal part of the junctional complex, are composed of transmembrane proteins of which occludin and the claudins are the best characterized, and peripheral membrane proteins like zonula-occludens 1 (ZO-1) which link the transmembrane tight junction-related proteins to the perijunctional cytoskeleton.[4-6] Initially regarded as static intercellular sealing structures, tight junctions are increasingly recognized as flexible gatekeepers regulating the flow across the epithelium.[7]

An abnormal barrier function, as reflected by increased intestinal permeability, has been implicated in a variety of conditions like Celiac disease and Type 1 Diabetes.[8-10] An increased intestinal permeability has also been shown to play a central role in the pathogenesis of Crohn's disease (CD) and ulcerative colitis (UC), jointly called inflammatory bowel disease (IBD).[11-16] IBD is a chronic, relapsing, inflammatory disorder of the gastrointestinal tract. It is a complex and polygenic disease that affects an increasing number of people in Western countries and more recently also in Asia.[17,18] The current accepted hypothesis for its pathogenesis is that a dysregulated immune response occurs to the commensal microbiota in the gut in a genetically susceptible subject.[19,20] It is thus plausible that an impaired intestinal epithelial barrier allows for the interaction between an unprocessed antigen and the mucosal immune system hereby leading to an abnormal immune response. It is however unclear whether the barrier dysfunction observed in IBD patients is a primary event or a consequence of the mucosal inflammation. Several studies suggest that an epithelial barrier dysfunction may have a primary role in triggering disease: IBD animal models have shown that increased intestinal permeability is a very early event in disease pathogenesis and precedes the development of disease.[21] Also, a persisting increased intestinal permeability was shown to be a risk factor for relapse in CD.[16] Finally, a proportion of unaffected first-degree relatives of IBD patients show an increased intestinal permeability.[12,13] First-degree relatives of patients with CD have the highest risk to also develop the disease. Although it is yet unclear if at-risk individuals with an abnormal intestinal permeability indeed go on developing the disease, there is an interesting case report of a young symptom-free girl who showed an abnormal intestinal permeability and whose brother had CD.[22] She had a negative workup thus showing no evidence of CD, but then went on to

*Correspondence to: Isabelle Cleynen;
Email: isabelle.cleynen@med.kuleuven.be
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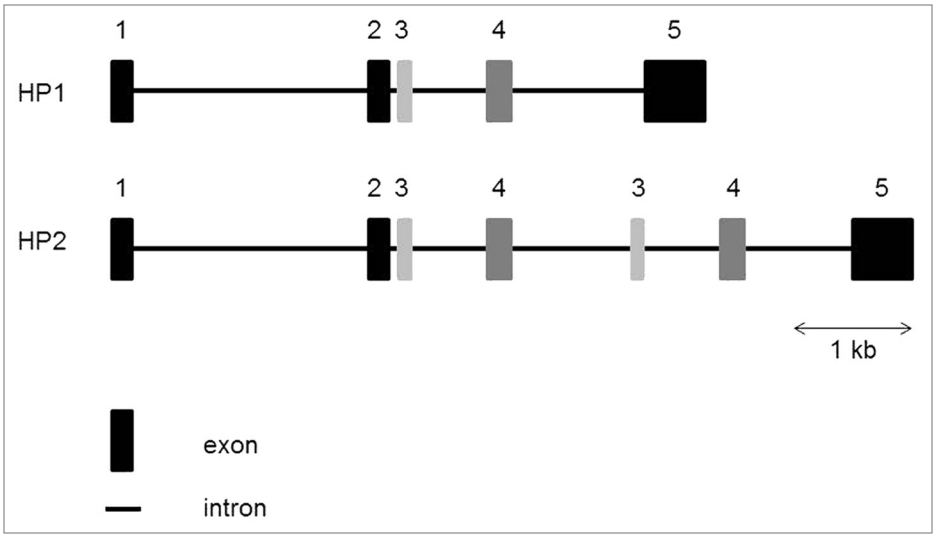

Figure 1. Structure of the human *HP* gene. The human *HP2* allele arose by duplication of exons 3 and 4 of the *HP1* allele. HP, Haptoglobin; kb, kilobase

develop CD 7 y later. This suggests a link between genetic susceptibility for the disease and abnormal intestinal permeability, and the subsequent development of the disease.

Zonulin and Haptoglobin

Structure and function of zonulin

In contrast to increasingly detailed knowledge about the structure of the tight junction, surprisingly little is known about the regulation of its permeability.

In a series of seminal papers, zonulin was identified as an important physiological regulator of paracellular intestinal permeability. This work started more than 20 y ago when Fasano and colleagues described zonula occludens toxin (Zot) as a second enterotoxin in *Vibrio cholerae*.[23,24] Zot was responsible for mild diarrhea in volunteers who were vaccinated with an attenuated *V. cholerae* strain in which the A subunit of the conventional cholera-toxin, encoded by c*txA,* was eliminated. The 45kDa Zot toxin is localized in the outer membrane of the bacterium and a 12kDa C-terminal fragment is secreted after cleavage at amino acid 288.[25] The biologically active sequence was localized to the first six amino acids of the newly-formed NH2-terminal part.[26] When the supernatans of the attenuated cholera strain or purified Zot was applied on rabbit ileum in Ussing chambers, a reversible decrease in transepithelial resistance was observed.[24,27] Similarly, administration of purified Zot during isolated loop perfusion in rabbits reversibly shifted intestinal fluid handling from absorption to secretion and increased the passage of large molecules like insulin, cyclosporine A, immunoglobulins and 4kDa PEG in the small intestine but not in the colon.[27-29] When co-administered with Zot, oral insulin effectively lowered blood insulin in diabetic BB/Wor rats with kinetics similar to subcutaneous administration suggesting increased paracellular passage.[29] The binding of the Zot in the ileum and jejunum showed a decreasing gradient from the villus tip to the crypt and was absent in the colon.[27,30]

Since Zot acted in a non-cytotoxic and reversible manner, it was serendipitously hypothesized that a eukaryotic analog of Zot may be operative in the (patho)physiological regulation of the tight junction. Using specific anti-Zot antibodies, a single protein with a molecular weight of 47kDa was detected in human intestinal tissue.[9,31] This human intestinal Zot analog was named zonulin due to its actions on the zonula occludens or tight junction. Similar to its prokaryotic analog, affinity-purified zonulin reversibly lowered the transepithelial resistance in the small intestine and not in the colon of non-human primates.[31] Moreover, the zonulin receptor was shown to be present on the apical surface only, since basolateral administration failed to induce alterations in intestinal permeability.

Comparison of the N-terminal end of zonulin and the active fragment of Zot[26] revealed a conserved common motif.[25,31] A synthetic octapeptide (GGVLVQPG), named FZI/0,[26,32] AT1001[33,34] and recently larazotide,[35-37] corresponding to the amino acids 8–15 of this fragment, did not affect permeability, measured as transepithelial electrical resistance of rabbit ileum in Ussing chambers.[26] However, pretreatment with AT1001 offered a significant protection against the effect of subsequent treatment with purified Zot or zonulin.[26,31] Conversely, a synthetic hexapeptide (FCIGRL), named AT1002, comprising the first six amino-acids of the active Zot fragment (aa 288–293), reproduced the effect of Zot and zonulin on paracellular permeability by increasing in vivo (quantified by a lactulose/rhamnose urinary excretion test after gavage of AT1002) and in vitro (transepithelial electrical resistance) permeability of murine and rat small intestine.[38,39] AT1002 is currently being studied for applications to enhance oral drug absorption.[28,40,41]

So far, gluten and bacteria (commensals and pathogenic) have been identified as triggers for small intestinal, luminal zonulin release from intact intestinal tissue and epithelial cell monolayers.[42,43] The effect of the bacterial strains on intestinal permeability correlated with luminal secretion of zonulin and could be blocked by AT1001 pretreatment.[42] The increased paracellular

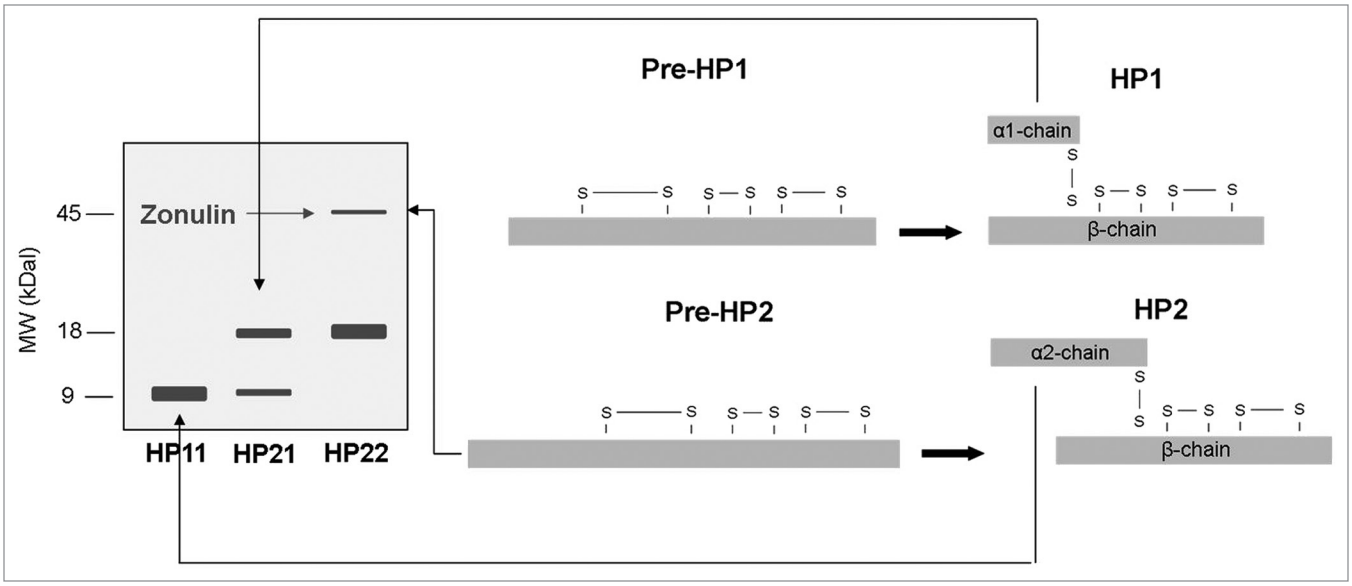

Figure 2. The structure of pre-HP1 and pre-HP2, and HP1 and HP2 is depicted on the right. It is also shown how the different proteins can be visualized on western blot (left side). (Adapted from[8]). HP, Haptoglobin; MW, molecular weight; kDa, kilo dalton.

permeability leads to increased intraluminal water secretion, possibly as part of the host innate immune response preventing bacterial colonization of the small intestine.[27,42]

The molecular mechanism through which zonulin enhances small intestinal permeability is still incompletely elucidated. In the initial report of Zot, a decrease in the number of freeze fracture strands and the number of strand intersections was shown, indicating a disassembly of the tight junction.[24] At the molecular level, Zot was demonstrated to induce polymerization of G-actin to F-actin in a protein kinase C α (PKCα) dependent manner, suggesting that contraction of the cytoskeleton is involved in the permeability-enhancing effect.[30] Follow-up studies demonstrated that Zot, zonulin and AT1002 operate through activation of the proteinase-activated receptor 2 (PAR2) and subsequent transactivation of the epidermal growth factor receptor (EGFR).[38,44] PAR2 is activated by proteolytic cleavage of the N-terminus of this G-protein coupled receptor, uncovering a tethered ligand which activates the receptor. The sequence of the active fragment of Zot (FCIGRL) is reminiscent of the activating peptide (SLIGRL) for PAR2, which has been shown to increase intestinal permeability through activation of PAR2 without receptor cleavage.[45,46] Recombinant zonulin and AT1002 did not alter permeability in *PAR2*-knockout mice or in Caco2-cells in which PAR2 was silenced.[38,47] Moreover, phosphorylation, i.e., activation, of EGFR was reduced in Caco2 cells with silenced *PAR2* upon stimulation with recombinant zonulin. Further, the barrier-disrupting effect of zonulin was blocked by an EGFR-specific protein tyrosine kinase inhibitor.[47] Recently, Goldblum et al. reported that this signaling cascade, activated by the prokaryotic zonulin-analog Zot, ultimately leads to a dissociation of ZO-1 from its transmembrane binding partners occludin and claudin-1 and its cytoskeletal binding partner myosin 1C in intestinal epithelial cells.[38] Moreover, ZO-1 and occludin were shown to be displaced from the tight junction. A similar mechanism can be hypothesized for zonulin, but these data are currently missing. Intriguingly, most of the data on PAR_2-activation and permeability were performed in the colon,[46] a region where zonulin is not active, and a recent paper failed to find an effect of jejunal PAR2-activation on paracellular permeability.[48] This apparent contradiction between the effect of zonulin and the distribution of its putative receptor PAR2 needs to be clarified in future studies. Further, it is unclear how the zonulin-regulated pathway relates to the recently described pathways of intestinal paracellular flux.[6,49,50] At least two different routes of intestinal paracellular flux are operative: a large-capacity pathway for small solutes (less than 4Å) and water, which was termed the pore pathway, and a small-capacity pathway for larger molecules, the leak pathway.[6] The pore pathway is mainly regulated by the expression of claudins, while passage through the leak pathway depends on phosphorylation of the myosin II regulatory light chain by myosin light chain kinase (MLCK) and subsequent contraction of the perijunctional actin-myosin ring. Although passage through the zonulin-regulated pathway involves molecules with a radius exceeding the dimensions of the pore pathway, preliminary reports suggest that MLCK is not involved since a reduction of pMLC was observed after AT1002 application on Caco2 cells.[39] In contrast to the light chain of myosin 2,[6] a phosphorylation of myosin1C was reported to be involved in the zonulin pathway.[38]

Structure and function of Haptoglobin

Recently, Fasano's group identified the molecular identity of zonulin as prehaptoglobin-2 (pre-HP2).[47] The gene coding for Haptoglobin (HP) is located on chromosome 16q22, and harbors a common polymorphism consisting of two structural alleles: *HP1* and *HP2*. HP2 is the product of a non-homologous intragenic duplication of exons 3 and 4 of HP1[51] (**Fig. 1**), resulting in three possible genotypes – *HP11*, *HP21* and *HP22*. HP can be

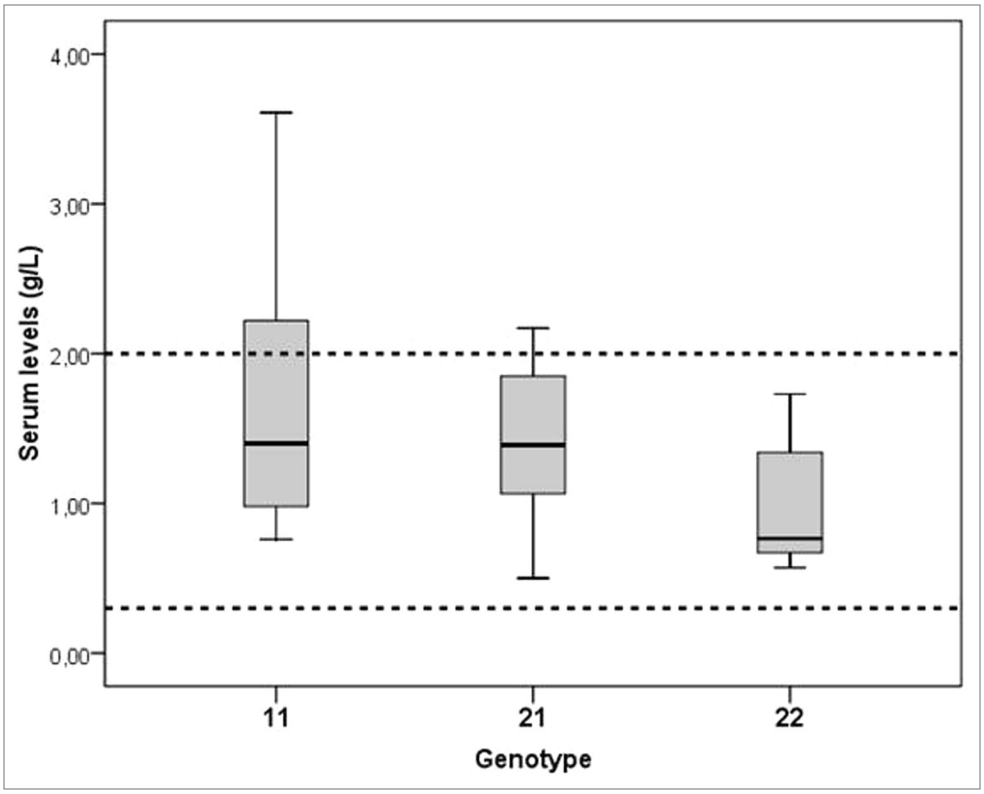

Figure 3. Haptoglobin serum levels in healthy controls for each *HP* genotype. Values represent medians (lines) with their quartiles (upper and lower level box).

detected in the serum of all mammals, but this particular polymorphism has been reported only in humans.[52] The three genotypes correspond to three different HP phenotypes. HP contains two types of polypeptide chains: a β chain, which is common to the three phenotypes, and an α1 or α2 chain, which differentiates HP11 (α1) from HP22 (α2). The three major phenotypes of HP are: an α1–β dimer for HP11; multiple α2–β units (cyclic polymer) for HP22; and a combination of α1–β dimer with multiple α2–β units (linear polymer) for HP21.[53,54]

Western blot analysis of human sera with anti-Zot primary antibody demonstrated three different bands: a 9kDa protein band, an 18 kDa protein band and a fainter 45kDa band. Through tandem mass spectrometry (MS/MS), the 9kDa band was identified as the α1-chain of HP1 and the 18 kDa band as the α2-chain of HP2. The ~45kDa protein, which was previously shown to represent zonulin,[31] was only present in individuals with a single 18 kDa band, i.e., homozygous for *HP2* (*HP22*). NH2-terminal sequencing and MS/MS analysis revealed the protein as pre-HP2.[47] **Figure 2** shows the structure of pre-HP1 and pre-HP2, and HP1 and HP2, and how the different proteins can be visualized on western blot. Tripathi et al. further substantiated the functional identity by producing pre-HP2 as a recombinant protein in a baculovirus expression system. Single-chain pre-HP2, but not the two-chain cleaved HP2, dose-dependently increased intestinal permeability when applied ex vivo in a murine small intestine indicator system and also in vivo, characterized by an increase in the lactulose-mannitol ratio in the urine after gavaging the respective sugars in pre-HP2 treated mice.[47] These series of experiments confirms the unusual feature of the protein precursor pre-HP2 exerting a completely different function as the mature cleaved HP protein.

HP is an acute phase α-sialoglycoprotein with hemoglobin-binding capacity. Binding to hemoglobin prevents iron loss and subsequent kidney damage during hemolysis.[55] Hemoglobin–HP complexes are removed by binding to the CD163 receptor, which is expressed on the surface of monocytes and macrophages.[52] Functional differences between HP1 and HP2 in their ability to protect against hemoglobin-driven oxidative stress have been shown to have important clinical significance. For example, *HP22* individuals with diabetes mellitus appear to be at significantly higher risk of microvascular and macrovascular complications.[56,57]

HP also has been demonstrated to modulate several aspects of the innate and adaptive immune response and to have anti-inflammatory activities.[58,59] Hp has the ability to suppress monocyte production of tumor necrosis factor (TNF), interleukin (IL)-10 and IL-12.[60] I*n vivo*, in a model of lipopolysaccharide (LPS)-induced bronchopulmonary hyper-reactivity and endotoxic shock, *Hp* knockout mice were more sensitive than their wild-type counterparts.[60] Also, a protective effect of Hp against experimental autoimmune encephalomyelitis, a model characterized by raised pro-inflammatory cytokines such as interferon (IFN)γ, IL-23, IL-12 and IL-17 in the brain, was shown.[58] Altogether, Hp seems to have a protective role in reducing the severity of Th1/17-mediated inflammatory processes.

HP is mainly produced by hepatocytes, and to a lesser extent by cells in other tissues such as lung, skin and kidney, under inflammatory conditions. Its synthesis is induced by IL-6, IL-1β and TNF.

In vitro studies have shown some functional differences between HP1 and HP2 protein products, with HP1 having a superior anti-inflammatory effect compared with HP2,[61-63] but the exact functional significance of this polymorphism is not well understood. As the *HP* polymorphism is only present in humans, it is difficult to directly link mouse and human data. Hp in mice is believed to resemble the human HP11, with over 90% homology with the human Hp1 allele.[64] Wild-type mice would thus behave more like HP11 in humans, although this remains speculative. To disentangle the difference between HP1 and HP2 in mice in the context of IBD it will be important to study mice genetically modified at the *Hp* locus to harbor either the *Hp1* or *Hp2* allele.[65]

Role of haptoglobin in IBD and other intestinal and immune disorders

Preclinical data

As mentioned above, Hp has been shown to have a protective effect against LPS-induced bronchopulmonary hyper-reactivity and endotoxic shock,[60] and against autoimmune encephalomyelitis[58] in mouse models.

Studies in mouse models also showed that Hp provides a protective effect against experimentally induced (by dextran sodium sulfate (DSS) and Oxazolone (Oxa)) colitis: *Hp* knockout mice have more weight loss and higher macroscopic and histological scores as compared with their wild-type littermates.[66] Hp thus plays an important modulatory and protective role in inflammatory colitis in experimental models. One way of action is by inhibiting the production of several cytokines (IL-17, IFNγ, TNF and IL-6 in DSS-induced colitis, and IL-13 in Oxa-induced colitis).[66] Furthermore, wild-type but not knockout serum inhibited IL-17 production, suggesting that *Hp* knockout mice preferentially develop Th17 immune responses and that Hp modulates Th17 cells.[66] However, these findings do not exclude the possibility that Hp also affects other steps in the inflammatory cascade.

Clinical data

Evidence from genetic studies

Given the immunomodulatory properties of HP, the gene has been studied as a candidate gene in inflammatory diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), primary sclerosing cholangitis (PSC) and diabetes mellitus type 2 (T2D), where the *HP22* genotype was shown to be over-represented.[52,67-69] Two smaller studies looking at the *HP* polymorphism in Crohn's disease have produced conflicting results[70,71]: Maza et al. found that *HP11* was significantly less common in CD, similar to what was found in other immune-based diseases. However, Papp et al. found a higher frequency of *HP11* in CD. In a well-powered study from our group, we found that *HP2* is a risk allele for IBD, with a higher frequency in both CD and UC compared with controls (**Table 1**). *HP2* was also over-transmitted to affected offspring in CD trios (affected child + parents). In UC trios, the over-transmission—although numerically present—lacked significance, probably because of the smaller size of this specific group. As stated above, murine Hp is believed to resemble mostly human HP1,[64] meaning that wild-type mice would thus behave more like Hp11 in humans. Mouse model studies showed that Hp knockout mice are more susceptible to experimentally induced colitis than their wild-type littermates[66] which would fit with the protective effect of the *HP1* allele as seen in IBD patients.

Table 1. Distribution of *HP* alleles and genotypes in healthy controls, CD and UC

HP status	Healthy controls, %	CD patients, %	UC patients, %
HP1	43.1	35.4	35.9
HP2	56.9	64.6	64.1
HP11	17.1	14.5	13.9
HP21	50.1	41.7	44.0
HP22	32.8	43.8	42.1

HP, Haptoglobin; CD, Crohn's disease; UC, ulcerative colitis

In Primary Sclerosing Cholangitis (PSC), a chronic liver disease characterized by inflammation and fibrosis of the intra- and extrahepatic bile ducts and often found as comorbidity in IBD, we found that *HP2* and *HP22* were more common in patients with this condition than in controls, as was also reported by Papp et al.[71] After exclusion of patients who also had IBD, *HP22* was still the most common among patients, but the differences between the genotypes were no longer significant, suggesting that either the differences observed were mainly driven by IBD or that the PSC-only study (n = 85 PSC patients) had insufficient power because of small groups.[66]

The *HP* locus was not picked up in any of the genome-wide association studies performed in IBD or in the more recently published CD genome-wide association meta-analyses.[72-75] This might be explained by the fact that this polymorphism is not a single-nucleotide polymorphism, but an intragenic duplication, which may not have been covered by the single-nucleotide polymorphisms selected on the 6q22 region. However, the region was present in two genome-wide linkage studies[76,77] and also in a genome-wide linkage study meta-analysis.[78]

Evidence from serological studies

In clinical settings, HP levels are used as a marker for hemolysis.[79] Serum levels of HP increase during inflammatory processes.[80] A study by Lubega et al. showed that although HP had poor predictive values to discriminate between patients with and without active disease, there was a significant difference in HP levels between active and inactive patients, both in CD and in UC.[81]

Interestingly, we found that serum levels of HP were significantly lower in healthy individuals with genotype *HP22* than in healthy individuals with genotype *HP11* and *HP21* (**Fig. 3**): HP11: 1.38 g/l; HP21: 1.38 g/l; HP22: 0.89 g/l. The majority of the samples however fell within the normal range (0.3–2 g/l). It is however possible that we underestimated the real differences between the genotypes: The different subtypes of HP have different molecular mass and different structures depending on the genotype, and these differences can affect serum levels. Moreover, the concentrations of HP22 in inflamed tissues may

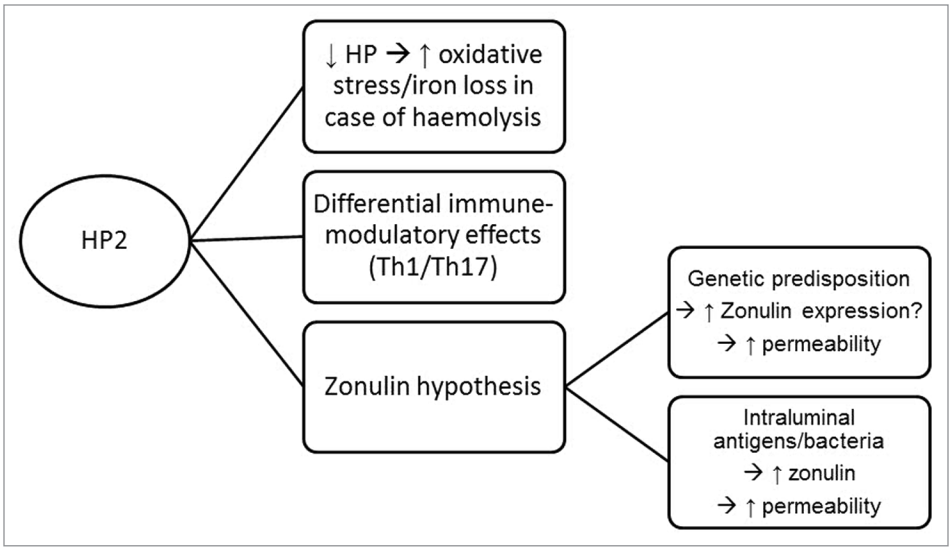

Figure 4. Summary diagram of the different ways HP(2) can be involved in IBD pathogenesis. HP, Haptoglobin.

also be lower because of the higher molecular mass and therefore lower tendency to leave the vessels.

Role of pre-haptoglobin (zonulin) in GI disorders

Celiac Disease

Celiac disease is an immune-mediated enteropathy, triggered by dietary gluten in genetically predisposed individuals.[82] Changes in intestinal permeability have been implicated in pathogenesis of celiac disease, but the underlying mechanism was unclear.[83,84] In the initial description of zonulin as the eukaryotic analog of Zot, increased zonulin protein concentration in patients with active celiac disease was reported.[9] They went on to demonstrate that exposure of IEC-6 intestinal cells to gliadin induced zonulin secretion and a AT1001-sensitive, PKCα-dependent actin polymerization and dissociation between ZO-1 and occludin, similar to what had been described for Zot and AT1002.[38,43] Paracellular permeability of rabbit small intestine mounted in Ussing chambers, was increased by adding gliadin to the mucosal compartment, but not when added to the serosal side. This effect of gliadin on permeability was abolished by AT1001 pre-treatment.[43] Similarly, gliadin induced zonulin release and higher permeability in vitro in biopsies of both healthy volunteers and patients with quiescent celiac disease.[85] However, both the baseline permeability and permeability after addition of gliadin were higher in patients with celiac disease and the luminal zonulin release was more pronounced and prolonged in patients. In follow-up studies, the chemokine receptor CXCR3 was shown to be the epithelial receptor for gliadin with subsequent recruitment of the adaptor protein MyD88.[86]

To confirm the contribution of zonulin to early permeability changes in celiac disease, 20 patients on gluten-free diet were randomized to a 3 d AT1001 treatment (12mg once daily) or placebo with a 2.5g oral gluten challenge on the second day.[34] Intestinal permeability was tested daily by a lactulose-mannitol urinary excretion test. In the placebo group a significant 70% increase in intestinal permeability was observed after gluten challenge in contrast to the AT1001 treated patients in whom no changes in permeability were observed. However, the difference between both groups failed to reach statistical significance. IFNγ increased in both treatment groups without a significant difference between groups. Leffler et al. evaluated a two week treatment with different doses of larazotide (AT1001), ranging from 0.5 to 8mg tid, with daily gluten challenge in 86 celiac disease patients on a gluten-free diet.[35] The primary endpoint, a difference in intestinal permeability, was not met in this study because of a large variability of the lactulose-mannitol ratios in this study. However, significantly less gastrointestinal symptoms were observed in patients in the active treatment arm.[35] In a second study from the same group, evaluating 1, 4 and 8 mg of larazotide tid vs. placebo in 184 patients with daily gluten challenge for 6 wk, gastrointestinal symptoms and anti-tissue transglutaminase IgA antibodies were lower in the active treatment groups. In contrast, and similar to the previous study, the urinary lactulose-mannitol ratio was similar in both groups.[87]

Taken together, these data suggest the involvement of zonulin in gluten-induced intestinal hyperpermeability in patients with celiac disease. Randomized clinical trials suggest that gluten-induced symptoms were reduced by the zonulin-antagonist larazotide. However, no effect on the lactulose-mannitol ratio, a measure for in vivo permeability, was observed. Whether this is related to shortcomings of the measurement technique or whether other effects apart from its effect on permeability play a role in the reduction of symptoms, remains to be demonstrated.

Inflammatory Bowel Disease

Clinical data on zonulin and IBD are currently not available. Arrieta et al. evaluated the effect of AT1001 in interleukin (IL) 10 knockout mice.[33] This model is characterized by increased small intestinal permeability,[88] preceding a chronic, patchy colitis which is dependent on luminal factors since germ-free animals are protected from disease.[89,90] AT1001 treatment started from weaning age dose-dependently reduced intestinal permeability. Moreover, colitis was prevented in the animals treated with high dose AT1001, in which small intestinal permeability was also

reduced and comparable to wild-type animals. To confirm that the conferred protection was not due an effect on colonic permeability, AT1002 was evaluated in wild-type and *IL10*$^{-/-}$ mice. AT1002 increased small intestinal permeability but did not affect the colonic barrier function, confirming earlier data on the distribution of the zonulin-receptor.[33]

Extra-intestinal disorders

The zonulin pathway has also been implicated in the pathogenesis of type 1 diabetes,[10,32,91] metabolic syndrome,[92,93] sepsis[94] and animal models of acute lung injury.[95] A detailed discussion of the extra-intestinal effects of zonulin is beyond the scope of the current review.

Conclusion

With the identification and confirmation of *HP2* as a risk allele for inflammatory bowel disease, the next question to answer is how HP affects disease pathogenesis. One possible explanation is linked to the immunomodulatory effect of HP. Based on mouse model studies it can be hypothesized that Hp has a role in reducing the severity of inflammation and modulating IL-17 production, a cytokine thought to be highly important in the pathogenesis of IBD. However, another hypothesis could be related to zonulin. With zonulin being a major physiological mediator of paracellular intestinal permeability, and its identification as pre-HP2, it is not unlikely that carriers of the *zonulin* gene (i.e., individuals with genotype *HP21* or *HP22*) could possibly have an increased risk to develop IBD, because of the permeating effect of zonulin on the intestinal barrier. Translational and functional studies addressing the role of zonulin in IBD, and its relation with intestinal permeability, will be important to further answer this intriguing question.

Figure 4 shows a summary diagram of the different ways HP(2) could be involved in IBD pathogenesis.

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