

Review

# Regulation of Antimicrobial Pathways by Endogenous Heat Shock Proteins in Gastrointestinal Disorders

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**Abstract:** Heat shock proteins (HSPs) are essential mediators of cellular homeostasis by maintaining protein functionality and stability, and activating appropriate immune cells. HSP activity is influenced by a variety of factors including diet, microbial stimuli, environment and host immunity. The overexpression and down-regulation of HSPs is associated with various disease phenotypes, including the inflammatory bowel diseases (IBD) such as Crohn's disease (CD). While the precise etiology of CD remains unclear, many of the putative triggers also influence HSP activity. The development of different CD phenotypes therefore may be a result of the disease-modifying behavior of the environmentally-regulated HSPs. Understanding the role of bacterial and endogenous HSPs in host homeostasis and disease will help elucidate the complex interplay of factors. Furthermore, discerning the function of HSPs in CD may lead to therapeutic developments that better reflect and respond to the gut environment.

**Keywords:** heat shock protein; Crohn's disease; inflammatory bowel disease; NOD2; immune homeostasis

## 1. Introduction

Crohn's disease (CD) is a chronic relapsing inflammatory disorder of the gastrointestinal tract and a major form of inflammatory bowel disease (IBD). The prevalence of CD differs worldwide, with Europe and Canada having among the highest rates at 322 and 319 per 100,000 persons, respectively [1,2]. While the precise cause of CD is unclear, it is widely accepted that disease arises from a complex interaction between genetic and environmental factors that disrupt normal host-microbe interactions in the gut.

The breakdown of intestinal mucosal barrier function is a key feature of CD pathogenesis. Intestinal epithelial cells play an active role in maintaining immune homeostasis by forming a barrier between the underlying tissues and the microbe-rich luminal environment [3]. The contribution of genetics in the pathophysiology of CD is demonstrated by numerous CD-risk associated polymorphisms in genes relevant to epithelial integrity and innate immune recognition [4]. There is also an expanding group of genes involved in monogenic IBD that are associated with significant intestinal epithelial barrier dysfunction [5]. However, the  $\geq 200$  susceptibility loci identified to date account for only 26% of CD variance [6]. This "missing heritability" in turn is determined by an estimation of genetic risk based on twin concordance studies, variants associated with gene expression

regulation rather than protein-altering variants, and a gene–environment/microbiome interaction that has proven difficult to model without a better understanding of environmental triggers [7–10].

Intestinal epithelial cells that are subjected to stress have the intrinsic ability to resist injury via various cytoprotective responses. Of these, endogenous heat shock proteins (HSPs) represent a major mechanism for protecting intestinal epithelial cell function and viability against a variety of environmental and physiological stressors. HSPs are found in all organisms from bacteria to humans and are among the most highly conserved proteins currently known [11]. HSPs are constitutively expressed at low levels but can be potently upregulated in response to a range of cellular stresses including nutrient deprivation, oxidative stress, inflammation, and infections [12]. As molecular chaperones, HSPs assist in the folding of newly synthesized polypeptides and assembly of multiprotein complexes, prevent abnormal protein folding or aggregation, maintain protein conformation to facilitate binding, mediate the intracellular protein trafficking, and degrade damaged proteins via the ubiquitin-proteasome pathway [13–15]. In addition to intracellular functions, HSPs can be released extracellularly where they activate immune cells by processing and loading peptide onto either class I or II MHC molecules through direct or cross-presentation [16].

In recent years, studies in CD patients have demonstrated interesting relationships between HSPs (both self and bacterial) and inflammation. CD patients with active disease display significantly increased intestinal HSP expression [17,18]. Therapies that cause overexpression, or alternatively down-regulation, of specific HSPs have been considered as potential supplemental therapies for CD treatment [19–23]. Nonetheless, self and bacterial HSPs could represent an important environmentally-regulated and disease-modifying factor in the development of different CD phenotypes. Here, we review the current understanding of HSPs in mediating host inflammatory responses in CD, and we describe ongoing studies of bacterial HSPs in CD pathogenesis and protection.

## 2. HSP Families

HSPs are categorized into six families based on their molecular weight: small HSPs (sHSPs), HSP40, HSP60, HSP70, HSP90, and large HSPs (LHSPs) (Figure 1).

### 2.1. Small HSPs

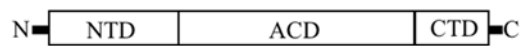
sHSPs are structurally defined by a conserved  $\beta$ -sandwich  $\alpha$ -crystallin domain flanked by non-conserved N- and C-terminal sequences [24]. While most prokaryotes contain only one or two *sHSP* genes, some pathogenic bacteria contain none and some symbiotic bacteria contain  $\geq 10$  *sHSP* genes [25]. In comparison to prokaryotes, sHSPs reportedly evolved independently in the main eukaryotic lineages, including animals, plants and fungi [26]. There are eleven ubiquitous sHSPs encoded by *HSPB* genes that range in size from 12–42 kDa, with the most prominent members being HSP10 and HSP27 that function in the mitochondria and cytosol/nucleus, respectively. Most sHSPs cooperate and co-assemble into large ensembles and, unlike large HSPs, function in an ATP-independent manner [27,28]. sHSP activity is regulated at the level of the oligomeric ensemble by balancing between an inactive and active conformation [29–31].

### 2.2. HSP40

HSP40, encoded by *DNAJ* genes, is the largest chaperone family containing 49 members that function in the cytosol or mitochondria [32,33]. All members of the family contain the conserved J domain usually present in the N-terminal region [34]. There are additional conserved regions, that are used to categorize the HSP40 proteins into three groups: Type 1 proteins (DNAJA) contain a J domain, a Gly/Phe-rich region, and cysteine-rich region with four zinc-finger motif repeats; Type 2 proteins (DNAJB) contain a J domain and a Gly/Phe-rich region; and Type 3 proteins (DNAJC) contain only a J domain [35–37]. HSP40 members primarily function as HSP70 co-chaperones [32]. HSP40 ATPase activity is essential for HSP70 protein activity as ATP hydrolysis converts HSP70 from an open state to

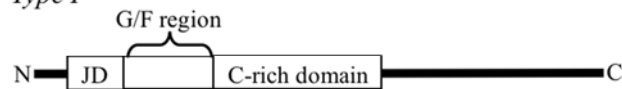
a close state [38]. In addition to its role with HSP70, HSP40 performs typical chaperone functions such as protein folding/unfolding, translocation, and degradation.

### Small HSPs (ex. HSP27)



### HSP40

#### Type I



#### Type II



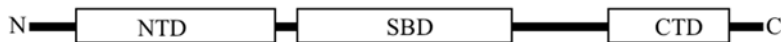
#### Type III



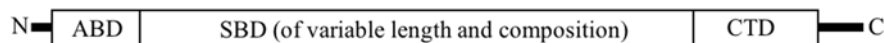
### HSP60



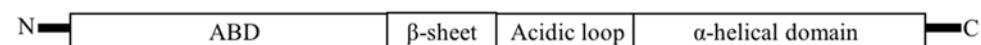
### HSP70



### HSP90



### Large HSPs



**Figure 1.** Major domains for each of the HSP families. NTD, N-terminal domain; ACD, α-crystallin domain; CTD, C-terminal domain; JD, J domain; G/F region, Gly/Phe-rich region; C-rich region, cysteine-rich region; E1/2, equatorial region 1/2; I1/2, intermediate region 1/2; AD, apical domain; SBD, substrate-binding domain; ABD, ATP-binding domain.

### 2.3. HSP60

HSP60 is an essential mitochondrial chaperonin encoded by the chromosomal gene *HSPD1* [32,39,40]. Most information on HSP60 structure is based on studies of the prokaryotic homolog, GroEL, which has three structural domains: apical (A), intermediate (I1/2) and equatorial (E1/2) [41]. As a mitochondrial protein, HSP60 catalyzes the folding of matrix proteins and maintains proteins in an unfolded state to channel them across the inner membrane for import or export [42,43]. HSP60 is

also secreted from cells and thus has a wide range of extracellular activities, such as stimulating human leukocytes and endothelial cells to produce a pro-inflammatory cytokine response [40]. Chaperonins differ from other ATP-dependent chaperones as they form a double ring structure stacked back-to-back with a central cavity for protein folding [44,45]. The chaperonin structure serves to prevent protein aggregation, which accelerates the rate of protein folding, although whether the mechanism is passive or active remains unclear [46,47].

#### 2.4. HSP70

The conserved HSP70 family, encoded by the *HSPA* gene family, are present in most prokaryotic and eukaryotic genomes [48,49]. The 13 proteins that comprise the HSP70 family are highly homologous with 52–99% amino acid identity [32]. HSP70 proteins are composed of three domains: a N-terminal nucleotide-binding domain with ATPase activity, a substrate-binding domain that interacts with stretches of hydrophobic amino acids, and an  $\alpha$ -helical C-terminal domain (the least conserved region) [50–54]. HSP70 proteins play indispensable roles as protein unfolding machines both in the cytosol and in membrane-bound organelles [48]. Furthermore, HSP70 proteins mediate the unpacking of endocytosed clathrin-coated vesicles and the translocation of organelle-targeted proteins to their proper translocase machinery [48].

#### 2.5. HSP90

The five members of the HSP90 family, encoded by the *HSPC1–5* genes, play critical roles in protein stabilization, function, and degradation. HSP90 proteins act as homodimers and consist of three conserved domains including an N-terminal ATP-binding site, a middle domain for activating ATP hydrolysis in the N-terminal domain and substrate binding, and a C-terminal domain for dimerization and substrate binding [55,56]. The number of HSP90 homologs per cell differs amongst organisms with bacteria containing one homolog called HtpG and yeast containing one to two homologs in their cytosol and mitochondria. Human cells containing four HSP90 isoforms including: cytosolic heat-shock inducible HSP90 $\alpha$ , cytosolic constitutively-expressed HSP90 $\beta$ , mitochondrial Trap1, and endoplasmic Grp94. Mediated by co-chaperones, HSP90 ATP hydrolysis is associated with conformational changes that moderate substrate interaction with proteins such as kinases, transcription factors, and steroid hormone receptors [57,58].

#### 2.6. Large HSPs

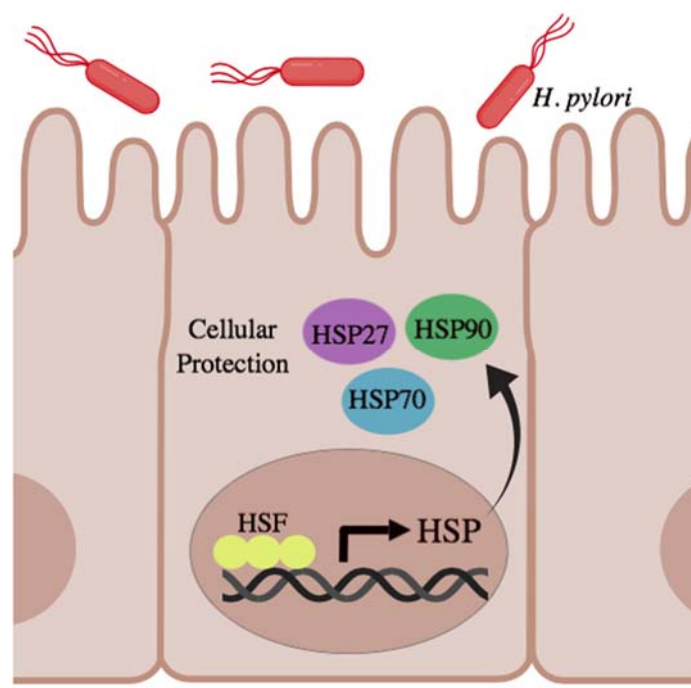
The family of IHSPs has two major members, cytoplasmic HSP110 (*HSP110* gene) and endoplasmic Grp170 (*HYOU1* gene) [59]. HSP110 has two different forms: 105 $\alpha$  which is constitutively expressed in the cytoplasm, and 105 $\beta$  which is localized in the nucleus and induced by heat-stress [60,61]. Grp170, also called Orp150 and HYOU1, is induced by a variety of stimuli including glucose starvation, hypoxia/anoxia, low pH, etc. [62,63]. IHSPs work with other chaperone proteins during periods of cytotoxic or proteotoxic stress [64,65]. IHSPs have an increased size due to an additional loop structure that aids in their ability to bind to polypeptide substrates or non-protein ligands [66].

As HSP are integral to many cellular functions, it is not unexpected that HSP polymorphisms are associated with, but not limited to, cardiovascular, neurological, and enteric disorders [67–71]. Moreover, heat shock transcription factor (HSFs) knockouts in mice have demonstrated various phenotypes including loss of thermotolerance, female/male infertility, reduced viability, partial embryonic lethality, and central nervous system defects [72]. In the next section, we review the role of HSPs in intestinal homeostasis and the polymorphisms associated with CD.

### 3. HSPs in Gastric and Intestinal Homeostasis

Aptly named cellular gatekeepers, HSPs play an essential role in the maintenance of gastrointestinal homeostasis by rendering epithelial cells in both the stomach and intestine more

resistant to stress or injury [73,74]. In response to epithelial disruption, HSFs are activated and translocated into the nucleus, where they bind to the promoter region of the *HSP* genes (Figure 2) [74].



**Figure 2.** Schematic of HSP induction following epithelial disruption by invading *H. pylori*. Upon epithelial cell disruption, activated HSF (in trimer) translocates to the nucleus, binds to the *HSP* gene promoter, and activates *HSP* gene transcription. HSPs then orchestrate various cellular protection mechanisms.

In mammalian cells, the stress response involves the induction of four major HSPs: HSP27, HSP60, HSP70 and HSP90.

To date, HSP70 (encoded by the *HSPA6* gene) polymorphisms have been observed in CD [71]. HSP70 polymorphisms perturb normal HSP70 function, enabling bacterial infection through a disrupted epithelial barrier [71,75]. The *HSP70-2* gene has various genotypes—AA, AB, and BB—due to a A–G transitions at the 1267 position that created a *Pst*I site [71,76]. The heterozygous genotype AB is the most commonly observed in CD-patients and A or AA genotypes, in comparison to BB genotypes, are associated with less severe CD [71,76–79].

More recently, Jostins et al. reported an IBD susceptibility locus on Chromosome 1 (with tagging SNP rs1801274) and prioritized the *HSPA6* gene through DAPPLE (Disease Association Protein-Protein Link Evaluator) and eQTL approaches [5]. Interestingly, mutations in *HSPA1L*, a member of the HSP70 family, have been reported in cases of CD in individuals with no family history of CD [71, 75,80]. Relative to HSP70, there is little known about how alterations in the function of other HSPs might impact CD pathogenesis. For example, HSP70 and HSP90 can function together to stabilize proteins, therefore further investigation is needed to determine how HSP polymorphisms impact their combined activities.

The induction of HSPs allows cells to survive otherwise lethal stresses, but this activity can also be subverted to support tumorigenicity and cancer cell resistance to therapy [81]. HSP overexpression has been reported for a wide range of cancers, including colorectal cancer (CRC), one of the most serious complications of CD responsible for approximately 15% of IBD-related deaths [82–84]. Overexpression of HSP27, for example, promotes carcinogenesis through multidrug resistance and inhibition of apoptotic cell death [85]. Conversely, a decrease in HSP expression is also associated with carcinogenesis. *Helicobacter pylori* (*H. pylori*), a known causative agent of gastric cancer,

reduces gastric epithelial cell proliferation by downregulating HSP70 expression through epigenetic modification [86,87]. HSP70 knockout mice have induced histological features of human IBD-associated colon cancer [88,89]. In attempts to inhibit HSP-induced carcinogenesis and tumorigenesis, researchers have utilized HSP-inhibitors or mutant HSPs to successfully sensitize human colorectal cancer cells to chemotherapy or radiation therapy [90–92]. Moreover, to combat *H. pylori*-induced gastric cancer, researchers have developed HSP complex vaccines containing HSP derived from *H. pylori*. Using a mouse mucosal vaccination model, HSP complex vaccines have been shown to induce protective immunity against *H. pylori* without the induction of a severe inflammatory response [93].

HSPs exemplify the goldilocks principle in which just the right amount of HSP activity maintains gastrointestinal homeostasis whereas too much or too little causes disease. In the following sections, we discuss some of the major mechanisms to regulate HSP activity and the role of bacterial HSP in human immunity.

#### 4. HSP Interactions with NOD-Like Receptor Innate Immunity Receptors

Mutations in the *NOD2/CARD15* gene are the strongest genetic determinants of CD susceptibility and severity identified to date. Individuals homozygous or compound heterozygous for *NOD2* variants have a 20–40-fold increased risk of developing CD [94]. *NOD2* (nucleotide binding oligomerization domain-containing protein 2) belongs to a family of intracellular pattern recognition receptors (PRR) called NLRs (nucleotide-binding domain and leucine-rich repeat containing receptors) and is expressed in peripheral blood mononuclear cells such as macrophages, granulocytes, dendritic cells and within intestinal epithelial cells, particularly Paneth cells. *NOD2* protein (as part of the innate immune inflammasome) is involved in several key processes of intestinal homeostasis including intestinal barrier integrity, microbial defense, innate immune regulation, oxidative stress, ER stress, and autophagy [95,96]. *NOD2* mediates intestinal homeostasis through intracellular sensing of bacterial muramyl dipeptide (MDP), a derivative of peptidoglycan. *NOD2* binding initiates innate immune responses via nuclear factor (NF)- $\kappa$ B, mitogen-activated protein kinase cascades, and caspase-1 leading to production of cytokines, mucins and anti-microbial peptides [94,96–99]. Mice deficient in *NOD2* have significantly increased susceptibility to colonization by pathogenic bacteria, putting them at risk for bacterial infections and intestinal inflammation [100–103]. *NOD2* is also a vital component of the inflammasome, a multiprotein inflammatory complex that detects invading pathogens and environmental stressors and is involved in several key processes of intestinal homeostasis including intestinal barrier integrity and microbial defense [104]. Mutations in NLRP3, another NLR and inflammasome component, are also linked to CD susceptibility [105]. Indeed, abnormal activation of the NLRP3 inflammasome directly affects intestinal inflammation in humans and in models of experimental colitis models [106–108]. Given the impact of dysfunctional *NOD2* signaling on intestinal homeostasis and CD, significant research attention has been devoted to understanding how *NOD2* activity is regulated.

To maintain cellular stability and avoid degradation, *NOD2* is constitutively associated with HSP90 [109]. HSP90 is believed to bind to a conserved protein domain on the *NOD2* protein called the NACHT domain [110]. In this state, *NOD2* is considered inactive, but signal-competent [104]. *NOD2* becomes active and begins signaling when HSP90 dissociates from *NOD2* due to MDP binding [109,111]. Following activation, *NOD2* is subsequently tagged with ubiquitin for degradation, an action believed to confer tolerance to MDP [109,110]. *NOD2* activity is further regulated through interaction with the substrate binding domain of cytosolic HSP70. Grimes et al. demonstrated that HSP70 overexpression increased and down-regulation decreased, the half-life of *NOD2* and therefore signaling duration in response to MDP stimulation [112]. HSP70 has also been shown to stabilize *NOD2* variants associated with CD (e.g., G908R, R702W, and 1007fs) and restore proper responsiveness to MDP [113]. As these CD mutants are still able to sense MDP, but have a diminished NF- $\kappa$ B response, restoring proper responsiveness is essential to maintaining gut homeostasis [114].



## 5. Environmental Factors That Influence HSP Abundance

HSPs are constitutively expressed under physiological conditions but expression can increase dramatically in response to four types of stimuli: (1) physical, including heat shock and radiation; (2) chemical; (3) microbial, such as commensal/pathogenic bacteria, viruses, parasites, and fungi; and (4) dietary. Physical and chemical stimuli have been extensively discussed in previous articles, thus we focus on microbial and dietary factors [115–119].

### 5.1. Microbial Stimuli

Physiological expression of inducible HSPs in the intestine is thought to be maintained through interactions with commensal microbes. HSP27 (or HSP25 in mice) and HSP70 are preferentially expressed in colonic epithelium as compared to the small intestine, which is exposed to relatively smaller amounts of microbes [120,121]. Furthermore, colonic HSP expression is reduced or absent in germ-free mice, indicating a requirement for microbial stimuli [122]. Multiple bacteria and bacterial products have been shown to induce HSPs in the colon, including lipopolysaccharide (LPS) and short-chain fatty acids (SCFAs) [123–126]. Some examples include soluble factors from probiotic bacteria, such as *Lactobacillus GG* and *Bifidobacterium breve*, which induce HSP25 and HSP72 [121,127–130]; LPS from *Escherichia coli* that induces HSP25 [124]; *Salmonella enteritidis* 857 that induces HSP70 and HSP90 [131]; and a sporulating factor from *Bacillus subtilis* that induces Hsp27 [132].

Oral antibiotics are commonly used to support immunosuppressive therapies for CD, with varying rates of success [133]. However, antibiotics can profoundly alter the composition and abundance of bacterial within the gut microbiome and have been shown to have a corresponding impact on the expression of intestinal HSPs [134]. Mice treated with broad-spectrum antibiotics such as metronidazole exhibit reduced intestinal expression of HSP25 and HSP72 and, in combination with ampicillin, neomycin, and vancomycin, increased mortality in a model of chemically-induced colitis [121,135]. These studies suggest that antibiotic therapy may disrupt physiological HSP induction, and affect intestinal vulnerability to infection and inflammation.

### 5.2. Dietary Stimuli

Food-derived nutrients and bioactive substances protect cells through mechanisms that involve the induction of HSPs during stress and/or fasting [136]. Food restriction is a stressful condition. Fasting increases HSP27 and HSP90, but not HSP70, in the gastrointestinal tract of piglets [137,138]. Conversely, glutamine, the most abundant free amino acid in the body, is a major substrate for intestinal cells and protects against cellular stress by inducing HSP70 expression [139]. Glutamine depletion can be associated with the metabolic stress associated with IBD and sepsis. Administration of glutamine improved survival in *HSP70<sup>+/+</sup>* mice in a sepsis model, but had no survival benefit in *HSP70<sup>-/-</sup>* mice [140]. Initial experiments using different individual amino acids, such as arginine, histidine, glutamate, proline, alanine, and glycine were unable to increase HSP concentration, although it should be noted that these experiments were tested in vitro, with the amino acid acted as the sole nutrient source [141]. Subsequent experiments, however, demonstrated many of these amino acids affected HSP levels. For example, arginine restored physiological levels of HSP70 in intestinal Caco-2 cells; glutamine induced HSP25 expression in rat IEC-18 cells; and threonine induced HSP25 and HSP70 in gastrointestinal epithelial cells [142–144]. In addition to the reported effects of free amino acids on gut epithelial HSP induction, dietary proteins and peptides also play a role in gut HSP expression. For example, consumption of whey protein increased HSP70 expression in the lungs and muscles of rats undergoing thermal stress from exercise [145].

Plant-derivatives are reported to have differential effects on HSP abundance in the gut. Plant lectins, for instance, are known to reduce levels of HSP70, HSP72, and HSP90 in the jejunum of rats [146]. Downregulation of HSPs in response to lectin is thought to contribute to severe disruptions in epithelial

layer integrity [146]. In contrast to lectins, psyllium fiber feeding increases HSP25, but not HSP32 and HSP70, expression in the jejunum, ileum, and colon [147]. Interestingly, the increased expression of HSP25 was not correlated with a change in HSF1 expression, suggesting that there may be a currently unknown mechanism of HSP regulation that does not involve HSF1 [147].

Phenols represent a diverse group of compounds that consequently have diverse functions in intestinal homeostasis. In early work using human colon cancer cell lines, several flavonoids, such as quercetin and kaempferol, were shown to inhibit the synthesis of HSPs during heat shock [148]. More recently, curcumin, another phenol, demonstrated a similar ability to reduce HSP70 levels in serum [149]. Using a sepsis rat model, Rocha et al. observed a decrease in HSP70 serum levels following curcumin dispersion seven days prior and two-days post sepsis induction [149]. In comparison to some flavonoids and curcumin, carvacrol induces HSP70 expression in the Peyer's patches of mice [150]. In this model, carvacrol amplified the CD4+CD25+FoxP3+ T cell recognition of HSP70 and downregulated inflammatory disease by suppressing proteoglycan-induced experimental arthritis [150].

Dietary factors can interact directly with host cells and indirectly through their effect on the gut microbiome [73,139,151]. Exclusive enteral nutrition (EEN) is a dietary intervention consisting of a liquid formula diet either composed of individual amino acids (elemental) or intact proteins (polymeric) that is used to induce remission in active CD [152–154]. EEN is a liquid formula diet either composed of individual amino acids (elemental) or intact proteins (polymeric) [152]. EEN therapy achieves remission in approximately 80% of pediatric CD patients [152,155]. While the exact mechanism for EEN therapy is unknown, EEN is thought to act through modifying microbial communities and microbial metabolite production in the gut [156,157]. As gut bacteria are important in the induction of HSPs during times of stress, EEN may not only affect the gut microbiome, but also gut bacterial induction of HSPs.

## 6. Role of Bacterial HSP in Host Immune Responses

Bacterial HSPs show varying degrees of homology to their eukaryotic counterparts but are highly conserved among pathogens [158]. For this reason, studies have observed bacterial HSPs to act as antigens, determined by increased levels of anti-heat shock protein antibodies, and inducers of humoral and cellular immune responses [159]. Bacterial HSPs have been shown to have both protective and pathogenic activities in the human host depending on the infection [160]. Three of the best studied bacterial HSPs in human health and disease include HtpG, DnaK, and GroEL.

### 6.1. HtpG

Animal infection models, in vitro cellular analysis, and human metagenomic studies have been used to study the function of HtpG, the bacterial homolog to the highly abundant chaperone HSP90. For certain bacterial genera including *Salmonella*, *Leptospira*, *Edwardsiella*, *Porphyromonas* and *Francisella*, HtpG appears to act as a virulence factor by aiding in pathogenesis and persistent infection [161–166]. For example, using a pig infection model of *Salmonella*, Verbrugghe et al. demonstrated the role of *S. typhimurium* HtpG in enabling bacterial persistence in intestine-associated tissues [162]. The activity of HtpG, however, is not always associated with disease. In a recent metagenomic study, Dunn et al. observed a decreased abundance of HtpG in CD patients compared to healthy controls [167]. Moreover CD patients unable to sustain remission had an even lower HtpG abundance than CD patients able to sustain remission [166]. The results from the Dunn et al. study suggest a role for bacterial HSPs in induction of mucosal tolerance as healthy individuals tended to have higher abundances of HtpG.

### 6.2. GroEL

GroEL is part of the HSP60 protein family and functions with assistance from the co-factor GroES. The GroEL/ES system has played an important role in the structural evolution of proteins by providing foldability and stability to proteins with otherwise deleterious mutations [168,169]. One of



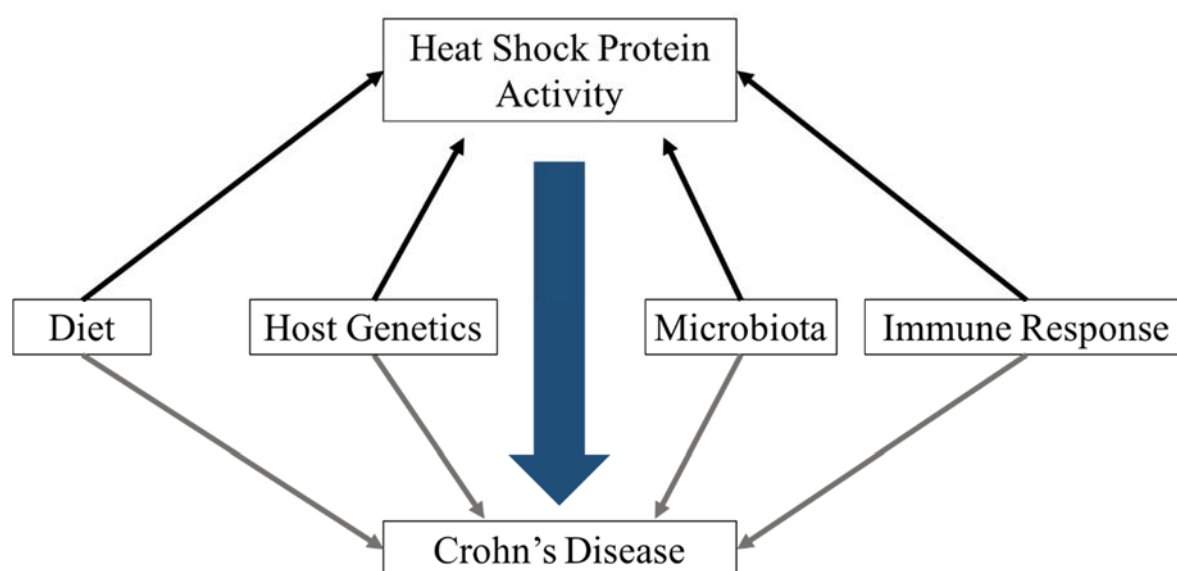
the major functions of GroEL is maintaining intestinal homeostasis by stimulating immunoregulatory pathways. A study by Ohue et al. demonstrated that GroEL caused naïve T cells to differentiate into CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup>T cells in the gut [170]. Moreover, Ohue et al. observed that mouse-derived HSP60 was not able to cause such differentiation suggesting that the production of Treg cells was dependent on HSP type. Similar to what was seen in the previous section with HtpG, GroEL from commensal bacteria appears to function as constitutive regulators of gut immune homeostasis. That being said, GroEL also plays an inflammatory role under specific situations. For instance, GroEL from *Aggregatibacter actinomycetemcomitans* and *Chlamydia trachomatis* induce human peripheral blood T-cell apoptosis, which is thought to manipulate the immune response [171–174].

### 6.3. DnaK

DnaK is the bacterial counterpart to eukaryotic HSP70. In a recent publication, Chuang et al. observed intranasal vaccination with DnaK derived from *Mycobacterium tuberculosis* resulted in protective immunity against tuberculosis in immunocompetent and immunocompromised mice [175]. Intranasal vaccination generated IFN $\gamma$ -secreting CD4<sup>+</sup> T cells in the spleen and IL-17-releasing CD4<sup>+</sup> T cells in the lungs [175]. The protective immunity generated by DnaK was comparable to the currently used BCG vaccine [175]. One year ago, Okuda et al. implicated DnaK in *Pseudomonas aeruginosa* translocation across the gut epithelium [176]. Inactivation of DnaK caused serious repression of *P. aeruginosa* through a colonic cell monolayer as well as decreased bacterial motility (swimming, swarming, and twitching), impacting bacterial colonization and spreading [176].

## 7. Conclusions

HSP function and expression are modulated by host genetics and environmental factors including gut microbes and their products and diet (Figure 3). HSPs play a key role in the maintenance of intestinal homeostasis by supporting host pathways critical to intestinal epithelial barrier integrity and innate immune recognition. Importantly, defects in these processes are closely associated with CD, suggesting that HSPs could provide additional insight to mechanisms of CD pathogenesis and treatment.



**Figure 3.** HSP activity and CD are influenced by many of the same factors including diet, host genetics, microbiota, and the immune system. For this reason, HSPs act as an additional important environmental factor that can modify the development of different CD phenotypes.

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