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The heat shock response and cytoprotection of the intestinal epithelium

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Abstract Following heat stress, the mammalian intestinal epithelial cells respond by producing heat shock proteins that confer protection under stressful conditions, which would otherwise lead to cell damage or death. Some of the noxious processes against which the heat shock response protects cells include heat stress, infection, and inflammation. The mechanisms of heat shock response–induced cytoprotection involve inhibition of proinflammatory cytokine production and induction of cellular proliferation for restitution of the damaged epithelium. This can mean selective interference of pathways, such as nuclear factor kappa B (NF-κB) and mitogen-activated protein kinase (MAPK), that mediate cytokine production and growth responses. Insight into elucidating the exact protective mechanisms could have therapeutic significance in treating intestinal inflammations and in aiding maintenance of intestinal integrity. Herein we review findings on heat shock response–induced intestinal epithelial protection involving regulation of NF-κB and MAPK cytokine production.

INTRODUCTION

The intestinal epithelium is exposed to an array of injurious agents ranging from pathogens like viruses or bacteria to their products, xenobiotics, chemicals, immune and inflammatory cytokines, and thermal and related stress stimuli. To some extent, it serves as a protective barrier between these agents and the sterile host environment. Exposure to such noxious stimuli may lead to a complex, but well-coordinated, signal transduction process to maintain intestinal integrity and function. The well-coordinated mechanisms result in increased proliferation of crypt cells, secretion of enzymes, and synthesis and secretion of immune and inflammatory cytokines and heat shock proteins (Hsps).

Following inflammation-inducing stimuli, such as pathogens or proinflammatory cytokines, a transcriptional activator of several genes, nuclear factor kappa B (NF-κB), is activated (Rogler et al 1998). Concurrently, the mitogen-activated protein kinase (MAPK) pathway can be activated. The activation leads to the expression of cytokine receptors, cell adhesion molecules, viral genes, and various inflammatory cytokines, including neutrophil chemoattractants that attract leukocytes to the respective sites to induce inflammation (Baldwin 1996; Hobbie et al 1997; Awane et al 1999; Martin et al 1999; Cario et al 2000; Yue and Mulder 2000). In addition, MAPK is activated by stress and growth factors that modulate the transcription of genes coding for protective and growth proteins leading to cellular proliferation and migration that are vital for restitution of the damaged epithelium. NF-κB is a critical regulator of the early pathogen response and an activator of the immune mediators. On the other hand, thermal stress induces the production of the putative Hsps through activation of the heat shock transcription factor (HSF). The Hsps produced protect cells against further injury by rescuing intracellular proteins from irreversible denaturation; hence the term ''chaperones'' (Wu 1995). Two groups of proteins, Hsps and proinflammatory cytokines, seem to operate antagonistically. Interestingly, anti-inflammatory cytokines that oppose the proinflammatory cytokines seem to work in favor of the Hsps for cytoprotection. Accumulating evidence reveals that Hsps suppress inflammatory gene expression and thereby inhibit the synthesis of inflammatory cytokines to curb inflammation. Blockade of NF-κB or MAPK-mediated inflammatory responses by Hsps or other agents can be of

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therapeutic significance. However, the actual mechanisms by which Hsps may act to suppress inflammatory cytokine production through these pathways are incompletely understood.

Because of the emerging significance of cytoprotection by intracellular mediators, we decided to review the possible roles of Hsps in regulating inflammatory pathways that may be significant for intestinal protection.

PROINFLAMMATORY CYTOKINE PRODUCTION

The NF-κB pathway

Although production of inflammatory cytokines in the intestinal mucosa is mainly a function of specialized cells of the immune system, such as the intraepithelial lymphocytes and other monocytes, the intestinal epithelial cells (IEC) are also involved in intestinal defense. They are known to produce an array of inflammatory cytokines either constitutively or after stimulation by pathogens such as viruses and bacteria, proinflammatory cytokines, ionization radiation, and chemicals such as phorbol myristate acetate (PMA) (Thanos and Maniatis 1995; Elewaut et al 1999). Most, if not all, of the produced inflammatory cytokines are mediated by the transcriptional activator NF-κB through the NF-κB pathway (Baeuerle and Henkel 1994) (Fig 1). The NF-κB is a p50-p65 Rel family protein heterodimer that transcribes various genes. The Rel family proteins are composed of 2 groups. One group consists of p50 (NF-κB1) and p52 (NF-κB2). This group has deoxyribonucleic acid (DNA)-binding and dimerization domains and a nuclear localization signal. The second group consists of p65 (Rel A), Rel (c-Rel), and Rel B. In addition to DNA-binding and dimerization domains, the second group is composed of transcriptional activation domains (Thanos and Maniatis 1995). The NF-κB normally occurs in its inactive form bound to the inhibitory kappa B (IκB) family proteins (IκB-α, IκB-β, IκB-γ, and BcI-3) in the cytoplasm. Activation of the transcriptional activity of the NF-κB requires the phosphorylation of IκB proteins and their subsequent degradation to generate the p50-p65 that translocates into the nucleus and activates the respective genes (Thanos and Maniatis 1995).

From the NF-κB–dependent cytokine production pathway, it can be deduced that blockade of this pathway at any point to inhibit its transcriptional activation reduces or arrests the production of the inflammatory cytokines and hence inflammation. Anti-inflammatory cytokines such as interleukin (IL)-10 and IL-4, nonvirulent bacteria such as *Salmonella* spp, intestinal bacterial fermentation products like short-chain fatty acids, and the heat shock response repress inflammatory cytokine production by abrogation of some steps in the NF-κB pathway (Schottelius et al 1999; Wu et al 1999; Neish et al 2000; Berin et al 2001).

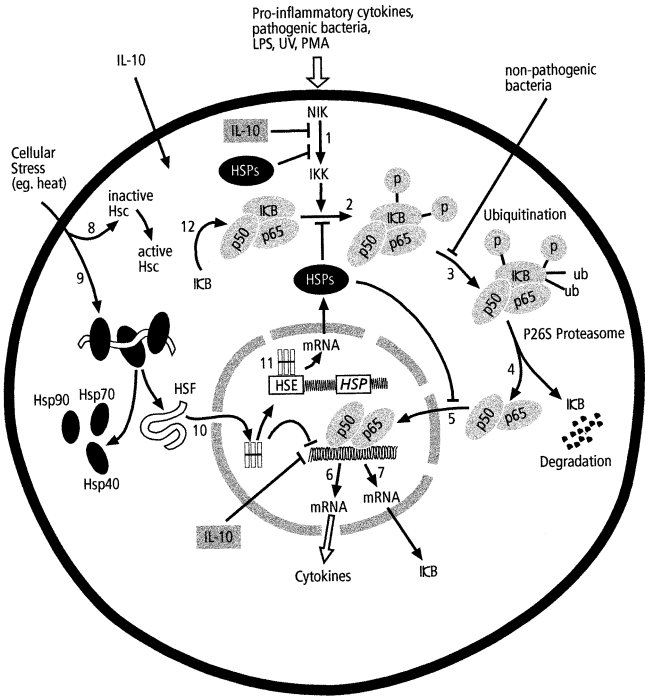

Fig 1. Cytokine regulation by NFκB pathway and the interaction with stress response. Inflammatory agents activate NIK that in turn activates IKK (1) to phosphorylate IκB (2). Phosphorylated IκB is ubiquitinated (3) prior to proteasome degradation of IκB (4) that releases free p50-p65. The heterodimer p50-p65 translocates into the nucleus (5) for transcriptional activation of inflammatory cytokines (6) and IκB (7). Stress stimuli acts on constitutive HSPs (8) and activates cytoplasmic bound inactive monomeric HSF (9) by freeing binding Hsps. HSF then translocates into the nucleus (10), trimerises and activates HSE to produce HSPs (11). Both inducible and activated constitutive HSPs may suppress cytokine production by inhibiting activation of IKK (1), stabilizing NFκB/IκB complex (2), or maintaining p65 in the cytoplasm (5). The HSF may repress expression of inflammatory cytokines (6). Anti-inflammatory cytokines may inhibit IKK activation (1) or inflammatory gene expression (6). Overexpression of IκB stabilizes the NFκB/I&kappa:B complex (12) to inhibit degradation. Non-pathogenic bacteria abrogate ubiquitination (3).

The MAPK pathways

Some cytokine secretions are signaled through the MAPK pathways that are known to transduce the extracellular stress signals. These pathways consist of extracellular signal–regulated kinases (ERK) 1/2, c-Jun N-terminal kinases (JNK) (also known as stress-activated protein kinases), and p38. Their activation is through cascades of MAPK, MAPK kinases, and MAPK kinase kinases that are in turn activated by the various extracellular stimuli (Fig 2). Inflammatory cytokines, such as IL-1β, IL-17, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ, pathogenic bacteria, such as *Salmonella typhimurium* and *Escherichia coli*, lipopolysaccharides (LPS), thermal and oxidative stresses, PMA, and growth responses can all activate the

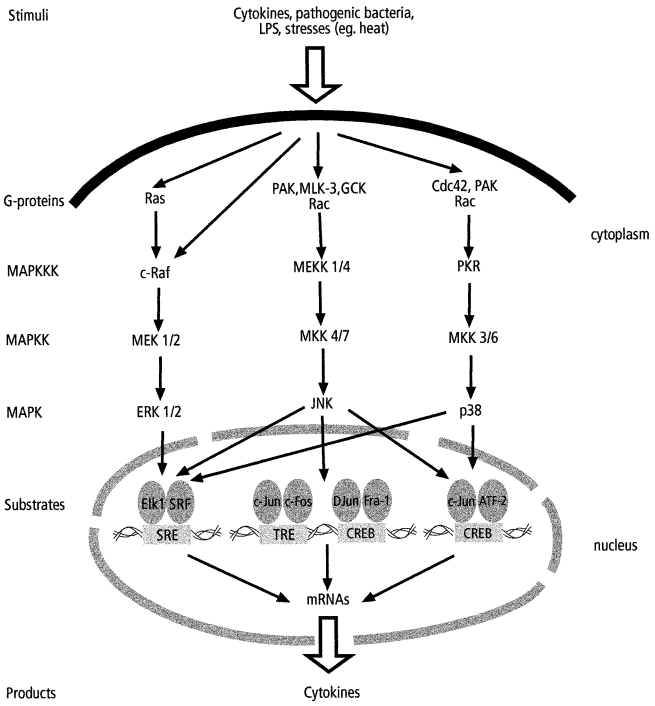

Fig 2. Cytokine production by MAPK pathways. Various inflammatory agents and stress stimuli activate MAPK pathways and their subsequent substrates leading to transcriptional activation of genes coding for cytokines. This activation is followed by production of an array of inflammatory cytokines. See text for details and Table 1 for summarized specific pathway stimulation and respective cytokine production.

MAPK-signaling cascades in the IEC, leading to the activation of genes coding for inflammatory cytokines (Hobbie et al 1997; Awane et al 1999; Martin et al 1999; Cario et al 2000; Yue and Mulder 2000; Czerucka et al 2001). Some of the cytokines mediated through MAPK in IEC are listed in Table 1.

THE ROLE OF THE HEAT SHOCK RESPONSE IN CYTOKINE REGULATION

The heat shock response and its protection of the IEC

Most cells and organisms react to heat and a variety of stressors by rapid synthesis of a group of evolutionary conserved proteins, ranging in size from 8 kDa to 150 kDa (Wu 1995; Ovelgönne et al 2000), termed Hsps. They are classified into several families according to their molecular weights. The major Hsp families include HSP150, HSP110, HSP90, HSP70, HSP60, HSP40, HSP20, and HSP8.5 (Table 2).

The heat shock response is induced by various stimuli, including thermal stress, heavy metals such as sodium arsenite and zinc ions, bacteria, and bacterial exo- and endotoxins, viral infections, ischemia, nutritional deficiency, ionizing radiation, oxidants, some IFN inducers, and cytokines in different cells including the IEC (Wu 1995). Following heat shock or other stresses, Hsps are produced after transactivation of the genes by a family of DNA-binding proteins called the HSFs (HSF1–4, of which the best known is HSF1). In unstressed cells the inactive HSF is bound to the cytoplasmic Hsp40 (Hdj-1), Hsp70, and Hsp90 in a monomeric form without the DNA-binding activity. In response to stress, HSF is released and translocated into the nucleus, where it assembles into a trimer and binds to a specific consensus heat shock regulatory element (HSE) in the heat shock gene promoter to exert the transcriptional activation (Santoro 2000; Han et al 2001) (Fig 3). Heat shock response also activates constitutive Hsps that, together with the induced Hsps, affect cytoprotection.

The various Hsp families are differentially expressed in the IEC and may relate to the type or intensity of the epithelial damage, or location of the IEC. Hsp60, Hsp72, and Hsp90 are expressed in the colonic mucosa after hyperthermia. In response to acetic acid–induced intestinal lesions, Hsp72 and Hsp90 inductions are protective. Their inductions precede that of Hsp60, which has no protective effect (Otani et al 1997). Although Hsp72 is induced by cellular injury, intestinal motility may be enough to induce Hsp60. Kuwabara et al (1994) and Sasahara et al (1998) demonstrated that water immersion stress that causes functional diarrhea without histopathological changes induces Hsp60 but not Hsp72 nor Hsp90 in both colon and small IEC. In these cases Hsp60 was observed to have no protective role against acetic acid–induced intestinal lesions. Hsp72 and Hsp73 have also been reported to have no protective function against small IEC indomethacin-induced injuries (Jin et al 1997). The chaperone function for a particular Hsp may, therefore, be specific to certain intestinal injuries or type and location of the IEC along the alimentary tract.

Cytokines involved in heat shock induction imply an interrelationship among these mediators. Because of their protective role, Hsps can be expected to down-regulate inflammatory cytokines to overcome inflammation. This suggests a mechanism of modulating cytokine production by both NF-κB and MAPK pathways. Several studies have shown that the heat shock response does, in fact, inhibit some cytokine production mediated by NF-κB and modulates MAPK-dependent cytokine production in a specific manner.

Hsps inhibit the NF-κB inflammatory cytokine production by IEC

There is accumulating evidence to suggest that Hsp induction abrogates the activation of the NF-κB inflammatory pathway and thereby inhibits proinflammatory gene expression (Cahill et al 1996; Chu et al 1997). Hsps ap-

Table 1 Cytokine production by IEC in association with activated MAPK pathways

Inducing agent	MAPK pathway	Function	Reference
IL-1β, IL-17, TNF-α	Ras-mediated JNK, p38, ERK1/2	Coactivation of NF-κB that produces CINC, MCP-1	Awane et al (1999)
IL-10	JNK	Early protection	Zingarelli et al (2001)
Absence of IL-10	Augmented JNK	Production of IL-6, TNF-α	Zingarelli et al (2001)
IFN-γ	Fas-mediated JNK	Production of IL-8	Martin et al (1999)
TGF-β	Ras-mediated ERK1/2, JNK	Production of TGF-β_1	Yue and Mulder (2000)
Salmonella typhimurium	ERK1/2, JNK, p38	Production of IL-8	Hobbie et al (1997)
EPEC	ERK1/2, JNK, p38	Production of IL-8	Czerucka et al (2001)
Stxs	p38	Production of IL-8	Thorpe et al (1999)
IL-1β	JNK	Production of IL-6	Hungness et al (2000)
LPS	ERK1/2, JNK, p38	Coactivation of NF-κB	Cario et al (2000)

EPEC, enteropathogenic *Escherichia coli*; Stxs, *E. coli* Shiga toxins; CINC, cytokine-induced neutrophil chemoattractant; MCP-1, monocyte chemoattractant protein–1; PMA, phorbol myristate acetate; IEC, intestinal epithelial cells; MAPK, mitogen-activated protein kinase; IL, interleukin; TNF, tumor necrosis factor; JNK, c-Jun N-terminal kinase; ERK, extracellular signal–regulated kinase; NF-κB, nuclear factor–κB; IFN, interferon; TGF, transforming growth factor; LPS, lipopolysaccharide.

Table 2 Major heat shock proteins (Hsps) known in mammalian cells

Family	Hsps	Function
HSP150	Hsp150	Molecular chaperone in ischemia and hypoxia
HSP110	Hsp110	Molecular chaperone in hyperthermia and ischemia
	Hsp105	Molecular chaperone in hyperthermia and ischemia
HSP90	Hsp100	Molecular chaperone for secretory proteins
	Hsp90	Molecular chaperone in hyperthermia and ischemia and for protein kinases. Translocation of cytosolic TNF-α and Raf kinases to the plasma membrane. Steroid hormone receptor functions. Cell proliferation and growth
HSP70	Grp78	Molecular chaperone in hyperthermia. Protein trafficking across the endoplasmic reticulum
	Hsp72	Molecular chaperone in hyperthermia, hypoxia, and ischemia. Inhibits leukotriene production
	Hsc70	Constitutive molecular chaperone for hyperthermia protection
	Hsp70	Highly induced molecular chaperone in hyperthermia and ischemia. Regulates the heat shock response
	mtHsp70	Mitochondrial Hsp70 molecular chaperone
HSP60	Hsp60	Molecular chaperone (chaperonin) in hyperthermia and ischemia
	Hsp65	Growth stress responses for tumor regression
	TCP-1	Molecular chaperone (chaperonin) in hyperthermia and ischemia
HSP40	Hsp47	Molecular chaperone for collagen
	Hsp40	Hsp70 cofactor for mediation of most Hsp70 functions
HSP20	Hsp27	Molecular chaperone in hyperthermia, chemicals (hydrogen peroxide, drugs), and irradiation. Actin protection. Enhances IL-10 production
	Hsp20	Molecular chaperone in hyperthermia, ischemia, and hydrogen peroxide

Grp, glucose-regulated protein; Hsc70, constitutive Hsp70; mtHsp70, mitochondrial Hsp70; TCP-1, T-complex protein; TNF, tumor necrosis factor; IL, interleukin.

pear to inhibit NF-κB transcriptional activation either by inhibiting IκB degradation or by directly repressing the NF-κB transcriptional activity (Chu et al 1997; Jobin et al 1999; Yoo et al 2000). Hsps are believed to prevent IκB degradation by inhibiting IκB kinase (IKK) activation, but how this occurs is unclear.

The normally occurring cytoplasmic IκB–NF-κB complex exists via an interaction between the IκB-α ankyrin domains and the nuclear localization sites. Human HSP70 has nuclear localization sites (Dang and Lee 1989). The presence of these nuclear localization sites raises the possibility that Hsps can specifically interact with the consensus IκB-α ankyrin domain and in turn hamper IκB–NF-κB phosphorylation and the subsequent IκB degradation (Yoo et al 2000).

There is strong evidence that Hsps enhance IκB production. In this case, elevated levels of IκB stabilize the IκB–NF-κB complex, resulting in hampered IκB degradation. Wong et al (1997) identified a 20-bp heat shock responsive segment in the human IκB-α that could be a functional heat shock responsive element in NF-κB transcriptional inhibition. In their study stress induced IκB-α messenger ribonucleic acid (mRNA) and protein expression, stabilizing the IκB–NF-κB association and suppressing the NF-κB transcriptional activation. Consistently, Pritts et al (2000) observed that heat stress was associated with the maintained IEC cytoplasmic IκB-α levels and the decreased endotoxin-induced NF-κB DNA-binding transcriptional activity. Both studies suggest dual mechanisms for NF-κB inhibition by heat shock response, increased expression of IκB-α and inhibition of the degradation of IκB-α.

Chaperones function by shielding the already synthesized proteins from degradation, mediating these stabi-

lizing effects through protein-protein interaction. Stabilization of IκB-α against phosphorylation and degradation following stress responses could partly be through this mechanism as well.

Though the mechanism is enigmatic, HSF acts as a transcriptional repressor of the cytokine genes. Cahill et al (1996) in their study on human monocytes showed that HSF represses the IL-1β gene responding to LPS by binding to a specific HSE in the IL-1β promoter. They suggested transcriptional repressor mechanisms that were distinct from those involved in the activation. In turn, this could block other cytokines that are secreted in response to intracellular IL-1β. This offers another potential mechanism for the down-regulation of cytokine expression by Hsps in IEC.

Hsps modulate the MAPK pathway to confer IEC protection

The protective role of Hsps in the IEC occurs through the modulation of the MAPK pathways. Hsps may selectively influence ERK1/2, JNK, and p38 MAPK pathways in various stressful conditions (Hill and Treisman 1995; Tilly et al 1996; Gabai et al 1998; Ng and Bogoyevitch 2000). Though most Hsps signal through the Ras-Raf–independent ERK1/2 MAPK pathway, the activation of the downstream genes may be specific. Hsp90 mediates normal IEC growth signals via ERK1/2, thereby protecting cells against apoptosis (Hostein et al 2001). Likewise, sodium arsenite induces Hsp70 synthesis in IEC via ERK1/2 activation of the HSF (Chen et al 2001). This Hsp70 together with Hsc70 suppresses JNK signaling, leading to cellular protection against various stresses (Mosser et al 2000). The JNK pathway has a potential to down-regulate IL-10 production to favor intestinal inflammation. Hence, its suppression by Hsp70 and Hsc70 is vital for anti-inflammatory responses. Interestingly, Hsp27 and Hsp72 prevent both repression of IL-10 production and induction of apoptosis by modulating the activity of the JNK. Subsequently, the accumulated Hsps suppress JNK to protect cells against stresses (Tilly et al 1996; Gabai et al 1998).

IL-6 production by the IEC after AP-1 activation by stress response is mediated through the JNK pathway. The stimulated JNK pathway activates c-Jun and c-Fos heterodimer members of AP-1 leading to IL-6 production (Andoh et al 1999; Yeh et al 2000). Though IL-6 is a proinflammatory cytokine, its production after heat shock induction could be protective. Barton and Jackson (1993) demonstrated a protective role for IL-6 against death from septic shock in mice. In their study IL-6 was observed to confer a significant reduction of the LPS-induced septic shock mortality.

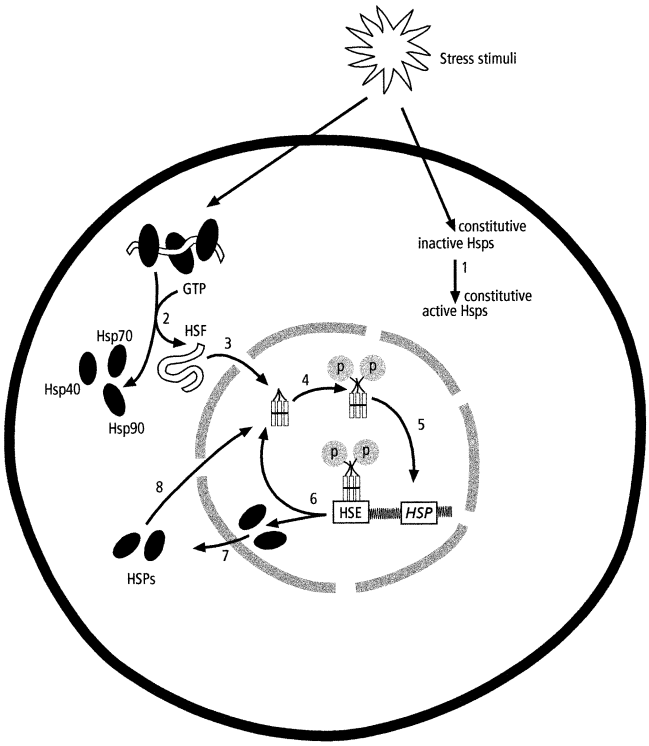

Fig 3. Regulation of HSPs. Stress stimuli activates inactive form of constitutive HSPs into active form (1). The inactive monomer non-DNA binding cytoplasmic HSF that resides bound to HSPs in unstressed cells is activated by stress stimuli and dissociates into HSPs and DNA-binding HSF (2). The active HSF translocates into the nucleus (3), trimerises and binds to HSPs gene promoter prior to undergoing phosphorylation at serine residues (4). Phosphorylated HSF attaches to the HSE located upstream of the HSPs genes (5) followed by transcription activation that results into production of HSPs and HSF (6). The HSF produced maintains the circle while HSPs are released into cytoplasm (7). High levels of cytoplasmic HSPs causes nuclear localization of HSPs (8) that in turn, bind to HSF to repress HSPs transcriptional activation.

CROSS TALK BETWEEN HSFs, NF-κB AND MAPK SIGNAL TRANSDUCTION PATHWAYS

Cellular responses at the gene level are highly conserved. The HSFs responding to establish cytoprotection after the heat shock response, the NF-κB for inducing inflammation and the MAPK responding to both protection and inflammation, seem to be coordinated in a very specific way. In this coordination the activation of the HSFs antagonizes the inflammatory activities of MAPK and NF-κB while favoring the anti-inflammatory responses. Similarly, inflammatory responses mediated by both MAPK and NF-κB seem to complement, if not support, each other.

Inhibition of the activities of the inflammatory cytokine TNF-α in the IEC is associated with the inhibition of the NF-κB and MAPK inflammatory responses. Interestingly, this modulation favors the protective, proliferative MAPK responses, induced by the epidermal growth factor, that

are important in epithelial restitution after injury (Kaiser et al 1999).

In other systems, an increase in the HSF DNA-binding activity and the subsequent Hsp production is associated with the enhanced protective influences of the ERK1/2 MAPK pathway and the suppressed JNK and p38 MAPK responses. Consistently, a decrease in the Hsp production is accompanied by an increase in the JNK MAPK activity that favors inflammatory responses. Absence of the protective ERK1/2 MAPK pathway blocks HSF transcription and Hsp up-regulation (Kim et al 1999; Tsuji et al 2000). Hsp protection of the IEC through the inhibition of JNK activation is also reported. Sodium arsenite induces Hsp70 in the IEC via the ERK1/2 MAPK pathway. This induction is associated with the HSF activation. Hsp70 together with Hsc70 produced through this pathway suppress the JNK pathway to confer cytoprotection (Mosser et al 2000).

The high constitutive levels of Hsp90 observed in unstressed cells (Buchner 1999) seem to play a vital role in modulating cellular protection through its interaction with other Hsps and various kinases. Recently, Hsp90 was found to be a repressor of the double-stranded ribonucleic acid–dependent protein kinase PKR, and its inhibition activates the kinase (Donze et al 2001). PKR is activated in response to viral infection, and it favors inflammation by interacting with IKK to catalyze NF-κB transcriptional activation (Zamanian-Daryoush et al 2000; Gil et al 2001). In addition, PKR activates MAPK p38 and contributes to LPS-induced IL-6 and IL-12 production (Goh et al 2000). These findings indicate a pivotal role of PKR linking NF-κB and MAPK that is controlled by Hsps. The phenomenon is such that the constitutive levels of Hsp90 suppress the PKR and thereby keep NF-κB inactive. Blockade of this Hsp not only activates PKR but also enhances the p38 activity and the ubiquitin-dependent proteasome degradation, a mechanism important for NF-κB activation (Schulte et al 1997).

The inhibition of Hsp production may also account for the decreased Hsp-augmented IEC production of IL-6 that is partly associated with the inhibition of the AP-1 activation (Hungness et al 2000). Inhibiting AP-1 activation suppresses inflammatory cytokine production even without inactivating NF-κB, indicating that blockade of either MAPK or NF-κB decreases inflammatory cytokine production. The production of both IL-8 and IL-6 depends on both pathways, and the inhibition of p38 MAPK and subsequent AP-1 activation abolish their production (Hobbie et al 1997; Hungness et al 2000). Interestingly, inhibiting NF-κB ubiquitin-dependent proteasome degradation by proteasome inhibitors activates HSF and AP-1 through p38 and JNK MAPK pathways (Tacchini et al 2001), resulting in a protective role. This implies a vital role for constitutive Hsps with regard to mediating signals to both MAPK and NF-κB pathways (Fig 4).

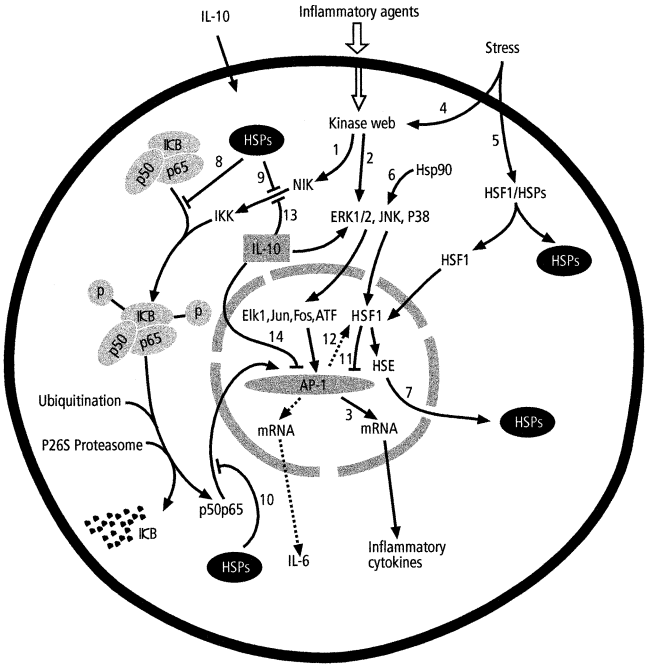

Fig 4. Interaction of stress and inflammatory responses. Inflammatory agents activate NF-κB (1) and MAPK pathways (2) through several kinases resulting into activation of AP-1 and subsequent production of inflammatory cyokines (3). Stress factors activate MAPK pathway through a series of kinases (4) and HSF1 (5) that translocates into the nucleus. The MAPK pathway is also activated by constitutive Hsp90 (6). Activated HSF1 binds to HSE followed by HSPs production (7) that block NF-κB pathway (8, 9, 10). The HSF1 may inhibit AP-1 inflammatory cytokine transcription activation (11). AP-1 has potential to activate HSF1 transcriptional activation (12). IL-10 blocks both IKK activation (13) and AP-1 activation (14) to repress inflammatory cytokine production. Dotted arrows represent stress response AP-1 products that are not produced by NF-κB. For more details see text.

These observations imply a tightly regulated communication network among various signal transduction pathways to elicit cellular and stimuli specific responses. HSF transcription and Hsp up-regulation is associated with protective MAPK activities mainly mediated through the ERK1/2 pathway. On the other hand, NF-κB inflammatory responses are connected with JNK and p38 MAPK pathways. More importantly, all 3 MAPK pathways can be activated at one time, but their responses are highly insulated from one another.

THERAPEUTIC SIGNIFICANCE

Selective blockade of the inflammatory NF-κB and MAPK pathways is important in reducing intestinal inflammations. Manipulations of these pathways are underway as an effective approach to inhibit proinflammatory gene expression. The principal mechanisms may be to activate

HSF and MAPK protective responses while inhibiting inflammatory NF-κB and MAPK pathways.

A number of studies suggest a close reciprocal relationship between the activities of HSF and NF-κB. In these studies the inhibitors of NF-κB activation that effectively prevented IκB-α degradation were potent activators of HSF transcription. Prostaglandins A and J were found to activate HSF and induce the synthesis of Hsps that protected cells against hyperthermia and virus infection (Santoro 1997). These prostaglandins were potent inhibitors of NF-κB transcription activation (Rossi et al 1997). Though their dual effects may neither be dependent nor be linked to one another, these findings strengthen the approach that maneuvering either of the pathways could be of therapeutic significance. Javadpour et al (1998) observed that a tyrosine kinase inhibitor herbimycin-A protected ischemic animals by inhibiting neutrophil infiltration, a sequel of the NF-κB–dependent chemokine synthesis. They suggested that the effect was because of the increased expression of Hsps by activated HSF.

The observations that enhanced ERK1/2 and suppressed JNK and p38 in association with up-regulation of Hsps confer protection against stress seem to be the principle underlying the working of some drugs. In such cases the absence of ERK1/2 that subsequently abolishes HSF transcriptional activation may render the drugs ineffective (Kim et al 1999; Tsuji et al 2000). By contrast, the activation of ERK1/2 by enteropathogenic *E. coli* via phosphorylation of the upstream Hsp54 enhances bacterial internalization (Czerucka et al 2000). Under such circumstances, prevention of phosphorylation of such Hsps may have therapeutic importance.

Advances in the direct inhibition of the inflammatory NF-κB or MAPK pathway without the involvement of Hsps are well reported. Potent inflammatory bowel disease drugs, mesalamine (5-aminosalicylic acid) derivatives, inhibit both inflammatory pathways (Kaiser et al 1999). Cytokine-suppressive anti-inflammatory drugs block the production of proinflammatory cytokines at a posttranslational level by binding to p38 and subsequently inhibiting its kinase activity (Lee et al 1994). Working together with NF-κB activation for cytokine production, inhibition of p38 could overcome both NF-κB and MAPK inflammatory responses (Hobbie et al 1997). Similarly, intracolonic introduction of single-stranded molecules of 15–25 bp (antisense phosphorothioate oligonucleotides) that can hybridize to the p65 mRNA and inhibit its expression has been shown to decrease the transcriptional activation of NF-κB. This medication lowers the synthesis of the proinflammatory cytokines IL-1, IL-6, and TNF-α and improves intestinal inflammation (Murano et al 2000).

Although inhibition of proinflammatory cytokines by the blockade of the NF-κB or MAPK pathway aids curb inflammation, long-term suppression of these pathways in humans or animals may not be a good idea to advocate for treating intestinal inflammations. The reasons partly stem from the findings by Inan et al (2000) and Erdman et al (2001) who showed high cytokine expression by the IECs of knockout and heterozygote mouse strains with less NF-κB. This may imply that NF-κB plays an important role in maintaining intestinal homeostasis and that other transcription factors can play a major role in inflammatory gene expression. In other cell systems prolonged inhibition of NF-κB results in massive cell death by apoptosis (Iimuro et al 1998).

CONCLUSIONS

The maintenance of the normal intestinal function at all situations is complex and involves many factors. These factors are regulated at multiple levels and interact with one another in a highly organized manner. Although NF-κB and MAPK pathways are involved in the production of inflammatory cytokines and cellular proliferation, their modulation by the heat shock response is beneficial for diverse harmful stimuli. Generally, the resulting Hsps not only protect cells by acting as chaperones but also down-regulate proinflammatory cytokine production to curb noxious processes, like heat stress, infection, and inflammation. In reality, the resulting heat shock response–induced cytoprotection may culminate in the restoration of the intestinal epithelial function.

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