Endoplasmic Reticulum Stress Induces Hyaluronan Deposition and Leukocyte Adhesion*

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There is mounting evidence that perturbations in endoplasmic reticulum (ER) function play a key role in the pathogenesis of a broad range of diseases. We have examined the ability of ER stress to modulate leukocyte binding to colonic and aortic smooth muscle cells. In vitro, control smooth muscle cells bind few leukocytes, but treatment with compounds that induce ER stress, including tunicamycin, A23187, and thapsigargin, promotes leukocyte binding. Likewise, dextran sulfate, another agent capable of inducing ER stress and promoting inflammation in vivo, strongly induces leukocyte adhesion. The bound leukocytes are released by hyaluronidase treatment, indicating a critical role for hyaluronan-containing structures in mediating leukocyte binding. Affinity histochemistry demonstrated that hyaluronan accumulates and is present in cable-like structures in the treated, but not the untreated, cultures and that these structures serve as attachment sites for leukocytes. Hyaluronan-rich regions of both murine and human inflamed colon contain numerous cells that stain intensely for ER-resident chaperones containing the KDEL sequence, demonstrating a relationship between ER stress and hyaluronan deposition in vivo. These results indicate that ER stress may contribute to chronic inflammation by forming a hyaluronan-rich extracellular matrix that is conducive to leukocyte binding.

The pathogenesis of chronic inflammatory conditions such as inflammatory bowel disease, atherosclerosis, and asthma is not well understood. During the immune response, leukocytes infiltrate and remain resident within the affected tissue, many in close proximity to smooth muscle cells (SMCs). Once the leukocytes leave the vasculature and enter the inflamed tissue, they encounter a myriad of extracellular matrix and cell surface components. However, it is not clear with which components the leukocytes interact nor what regulates leukocyte migration and adhesion within the inflamed tissue (1–3). Even less is known about the factors that determine the ultimate destination of migrating leukocytes.

Hyaluronan (HA) is a linear glycosaminoglycan composed of alternating units of β1,3-d-glucuronic acid and β1,4-N-acetyl-d-glucosamine (4). A family of hyaluronan synthase enzymes (Has-1, Has-2, and Has-3) (5) catalyze the formation of HA at the cell surface, and during synthesis, the nascent chain is extruded into the extracellular space. HA can associate with other extracellular matrix components and become incorporated into the matrix via high affinity interactions (4), or it can remain associated with the cell surface via several receptors, including specific forms of CD44 (4).

HA has several functions relative to inflammation, and the interactions of leukocytes with HA may be involved in their capture and retention within areas of inflammation. Hyaluronan accumulates in the extracellular matrix and in serum in many inflammatory states, and often the serum level correlates with the degree of inflammation (6, 7). Several studies have demonstrated that activated leukocytes can bind to endothelial cell HA (8–12), and we recently demonstrated that leukocytes can bind to virally infected SMCs via novel structures formed by HA (13, 14). The interactions of leukocytes with HA produced by SMCs may be important for modulating and perpetuating the inflammatory response; however, little is known about what factors regulate SMC production of the leukocyte-binding structures of HA. Identification of mediators that contribute to the production of HA as well as factors that can inhibit its accumulation may lead to the development of novel therapeutic targets and improved therapies for patients with chronic inflammatory conditions.

The endoplasmic reticulum (ER) is a dynamic membranous organelle with diverse functions including 1) protein synthesis, modification, folding, and subunit assembly; 2) steroid synthesis; 3) lipid synthesis; 4) glyoxgen production; 5) sequestration of calcium; and 6) maintenance of calcium homeostasis. Many infectious agents, environmental toxins, and adverse metabolic conditions interfere with ER function and homeostasis, thereby inducing ER stress (15–19). Cells elicit the ER stress response/unfolded protein response in an attempt to restore homeostasis by eliminating the discrepancy between ER capacity and demand (15–19). It is not known how the extracellular matrix is affected by ER stress.

Bertolotti et al. (20) recently demonstrated that perturbations in ER function that result in ER stress influence the development of dextran sulfate-induced colitis. Knockout mice deficient in Ire1α, a protein that senses and responds to ER

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U937 cells were grown in RPMI 1640 medium supplemented with 5% HEPES (Invitrogen), 10% fetal bovine serum (FBS) (Bio-Whitaker, Walkersville, MD), and an antibiotic/antimycotic mixture (Invitrogen). This demonstrates a novel alteration of the composition and function of the extracellular matrix as a consequence of ER stress.

EXPERIMENTAL PROCEDURES

Reagents—All materials were purchased from Sigma unless stated otherwise. The tunicamycin was from Streptomyces sp. The dextran sulfate for in vitro studies (sodium salt, molecular mass 36,000–50,000 daltons) was purchased from ICN Biomedicals Inc. (Aurora, OH). The mouse anti-KDEL antibody was purchased from Stressgen Biotechnologies Corp. (Victoria, Canada), the mouse anti-versican was from Seikagaku America (Palmouth, MA), and the rabbit anti-α-t-trypsin inhibitor was from DakoCytomation (Carpinteria, CA). All tissue was collected in accordance with an institutional review board-approved protocol from the Cleveland Clinic Foundation.

Cell Isolation and Culture—Human mucosal SMCs were obtained enzymatically from resected colon as described (13, 21). Rabbit aortic SMCs were obtained from thoracic aorta as previously described (22). Cells were grown in Dulbecco’s modified Eagle’s/Ham’s F-12 medium with HEPES (Invitrogen), 10% fetal bovine serum (FBS) (Bio-Whitaker, Walkersville, MD), and an antibiotic/antimycotic mixture (Invitrogen). U937 cells were grown in RPMI 1640 medium supplemented with 5% FBS and the antibiotic/antimycotic mixture. Cultures were maintained at 37 °C in 95% air and 5% CO2.

Leukocyte Adhesion—The quantification of leukocyte adhesion to SMCs was done as described (13). Briefly, SMCs were grown to confluence in 24-well plates and then treated with the appropriate test compounds for 23 h prior to the assay unless stated otherwise. U937 cells or freshly isolated peripheral blood mononuclear leukocytes (13) were employed significantly increased trypan blue staining. Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling analysis was employed to quantify apoptosis. Confluent cells were treated with test compounds and then trypsinized. The cells were rinsed in PBS, fixed in 1% paraformaldehyde for 15 min at 4 °C, rinsed again in PBS, resuspended in 70% ethanol, and then end-labeled. Apoptotic cells were detected by flow cytometry.

Dextran Sulfate-treated Mice—10–11-month-old FVB mice were employed in these studies, and all protocols were approved by the Cleveland Clinic Foundation IACUC. Dextran sulfate (sodium salt, average molecular mass 10,000 daltons; Sigma) was administered orally by adding it to the drinking water at a final concentration of 5%. After 3 days, mice were sacrificed, and their colons were removed, fixed in Histochoice, and embedded in paraffin.

Statistics—p values were calculated with Quattro Pro for Windows using a two-tailed Student’s t test. Differences were considered significant at p < 0.05.

RESULTS

ER Stress Induces HA-mediated Leukocyte Adhesion in Vitro—The effect of ER stress on colonic SMCs was examined by treatment for 23 h at 37 °C with or without tunicamycin, a well established inducer of ER stress (17, 19, 23). The SMC cultures were then incubated with radiolabeled U937 cells (a monocytic, leukemic cell line) for 1 h at 4 °C to allow attachment. Untreated, control SMCs bound few monocytes, but tunicamycin treatment dramatically induced leukocyte adhesion (Fig. 1A) with 5 μg/ml tunicamycin, resulting in optimal binding (2.8 × 10^6 leukocytes/well). The majority of bound leukocytes were removed with hyaluronidase treatment (Fig. 1A), demonstrating that HA has an active role in mediating the attachment. Likewise, pretreatment of the SMCs with hyaluronidase prior to the addition of leukocytes prevented their attachment (Fig. 1A). The hyaluronidase digestions were equally effective when the hyaluronidase was preincubated for 10 min with a mixture of protease inhibitors (aprotinin, bestatin, leupeptin, E-64, and pepstatin A), indicating that any contaminating proteases were not responsible for the observed effects. Human peripheral blood mononuclear cells also bound to tunicamycin-treated SMCs in the same HA-dependent manner (not shown). Immunohistochemical staining of the control and tunicamycin-treated cells demonstrated that tunicamycin did indeed induce ER stress, as evident by the intense intracellular staining for ER proteins containing the KDEL retention sequence (Fig. 1B). Tunicamycin also stimulated the deposition of large amounts of HA, and numerous thick HA cables were observed (Fig. 1B). Many small strands originating from cellular coats of HA coalesced into these larger diameter cables. Some but not all of the cables also stained positive for the hyaladherins versican and inter-α-trypsin inhibitor (IaI) (Fig. 1C). Transferase-mediated dUTP nick labeling assays

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2 Lesley, personal communication.
demonstrated that the tunicamycin treatment did not induce apoptosis under the conditions employed (1.4% of cells were apoptotic as compared with 1.5% of untreated, control cells). Together, these results demonstrate that tunicamycin treatment of SMCs promotes ER stress, the deposition of HA, and leukocyte adhesion by a mechanism involving HA.

Disturbances in calcium homeostasis also induce ER stress. A23187, a calcium ionophore, and thapsigargin, an inhibitor of the sarcoendoplasmic reticulum Ca2+/H1 ATPase type 2, are both potent inducers of ER stress. At optimal concentrations (2 and 0.5 μM, respectively), they also induced HA-mediated leukocyte adhesion to SMC cultures (Fig. 2A). In contrast, neither oxidant stress (250 μM H2O2 treatment for 23 h) nor heat shock (42 °C for 30 min and then 37 °C for 22.5 h) promoted HA-mediated leukocyte adhesion (Fig. 2B).

Dextran sulfate, a compound used in vivo to induce intestinal

Fig. 1. Tunicamycin induces monocyte attachment to SMCs via up-regulation of hyaluronan deposition. a, human mucosal SMCs were treated with or without 5 μg/ml tunicamycin for 23 h at 37 °C. The medium was then removed, and [35S]Cr-U937 cells were allowed to adhere for 1 h at 4 °C. Nonadherent cells were removed, and the SMC layer with adherent U937 cells was solubilized. In some wells, the cell layer was treated with hyaluronidase after the attachment of

Fig. 2. Induction of monocyte adhesion by disruption of calcium homeostasis but not by heat shock or oxidant stress. a, aortic SMCs were treated with or without 2 μM A23187 or 0.5 μM thapsigargin for 17 h. Radiolabeled U937 cells were added, and the cultures were incubated at 4 °C. b, aortic SMCs were heat-shocked (42 °C for 30 min) or exposed to oxidant stress (250 μM H2O2 for 23 h) and then pretreated with a blocking antibody against vascular cell adhesion molecule-1 prior to the addition of leukocytes. Adherent cells were quantified as described in the legend to Fig. 1. Data represent the mean ± S.D. n = 6; *, p < 0.05.
inflammation in a well-studied mouse model of colitis, also strongly promoted adhesion of leukocytes to SMCs (Fig. 3A).

Optimal adhesion was achieved by exposing SMC cultures to only 5–10 μg/ml dextran sulfate for 23 h. Some wells were treated with hyaluronidase after (Post H\text{ase}) or before (Pre H\text{ase}) the addition of U937 cells. Leukocyte attachment was assayed as described in Fig. 1. n = 6; *, p < 0.05; b, colonic SMCs were treated with dextran sulfate for 23 h and stained for HA (green) and nuclei (blue). Original magnification was ×10.

Fig. 3. Dextran sulfate induces hyaluronan-mediated leukocyte adhesion. a, colonic SMCs were treated with or without 5 μg/ml dextran sulfate for 23 h. Some wells were treated with hyaluronidase after (Post H\text{ase}) or before (Pre H\text{ase}) the addition of U937 cells. Leukocyte attachment was assayed as described in Fig. 1. n = 6; *, p < 0.05; b, colonic SMCs were treated with dextran sulfate for 23 h and stained for HA (green) and nuclei (blue). Original magnification was ×10.

ER Stress Has a Prolonged Effect on Leukocyte Adhesion—The most dramatic effects on leukocyte adhesion were observed when aortic SMCs were treated with dextran sulfate. Dextran sulfate (10 μg/ml) increased leukocyte adhesion 23-fold after a single 24-h exposure (Fig. 4A). When the medium containing dextran sulfate was removed after 24 h and replaced with regular medium, the SMCs remained adhesive for leukocytes for an additional 3 days (Fig. 4A). 24 and 48 h after the dextran sulfate was removed, the treated SMC cultures bound 6- and 5-fold more leukocytes, respectively, than control, untreated SMCs (Fig. 4A). By 72 h, the adhesiveness of the treated SMCs was still 40% greater than that of untreated, control SMCs (p < 0.05), but by 96 h, the treated SMCs were no longer adhesive (Fig. 4A). Also, we investigated whether the effect of dextran sulfate on leukocyte adhesion was sustainable or rapidly down-regulated in the continued presence of dextran sulfate. Aortic SMCs were treated daily with dextran sulfate for 5 days. After a single treatment, leukocyte adhesion was increased 23-fold (Fig. 4B). After 2 days of treatment, the adhesiveness for leukocytes increased to 95-fold above control levels. In the continued presence of dextran sulfate, the SMCs remained adhesive for 5 days, increasing to a maximum adhesiveness of 97-fold above control levels. For the entire 5 days, the adhesion was mediated by HA, as demonstrated by the capacity of hyaluronidase to remove the bound leukocytes (Fig. 4B).

HA Deposition Does Not Require de Novo Protein Synthesis—We next determined whether de novo protein synthesis is required for the induction of leukocyte adhesion to SMCs treated with dextran sulfate. Postconfluent aortic SMCs were pretreated with 25 μg/ml cycloheximide for 1.5 h and then treated with or without both dextran sulfate (10 μg/ml) and 25 μg/ml cycloheximide for 12 h. Dextran sulfate was equally effective at promoting leukocyte adhesion in either the presence or the absence of cycloheximide (Fig. 4C). Surprisingly, cycloheximide alone was also able to significantly induce leukocyte adhesion (Fig. 4C).

Unlike colonic SMCs, untreated aortic SMCs contained large amounts of intracellular HA, as indicated by its resistance to hyaluronidase digestion (Fig. 5A). Short strands of extracellular HA were observed in cultures of aortic SMCs after only 1 h of treatment with dextran sulfate (Fig. 5A). After 4 h, numerous fine HA-containing strands were observed (Fig. 5A). Such a rapid response suggests that new protein synthesis is unlikely to be required for HA cable production, consistent with the observation that cycloheximide does not block the effects of dextran sulfate on HA-mediated leukocyte adhesion. In fact, cycloheximide alone directly induced the rapid deposition of HA cables. After 2 h of cycloheximide treatment, small HA strands were present, and after 4 h of treatment, large cables were formed (Fig. 5A). The HA deposited in strands after only 1 h of treatment with dextran sulfate is capable of binding leukocytes (Fig. 5B). After 2 h of treatment, numerous leukocytes can be bound to the strands, and by 4 h even large cables bound with leukocytes are present (Fig. 5B). Following 24 h of treatment, many large cables are present that mediate leukocyte adhesion (Fig. 5B). Quantitative leukocyte adhesion assays support these findings and demonstrate that leukocyte adhesion increases with increasing treatment times. Maximal binding was observed after 23 h of dextran sulfate treatment, and after 1, 2, and 4 h of treatment, 40, 46, and 49% of maximal binding was observed, respectively.

ER Stress Is Associated with HA Deposition in Vivo—Finally, we investigated the association between ER stress and HA deposition in vivo. For our first approach, we examined mice treated orally with dextran sulfate to induce inflammation in the colon. Colonic tissue from mice treated with dextran sulfate for only 3 days showed intense staining for both HA and KDEL-containing proteins, whereas tissue from untreated mice had only minimal staining for HA, and low levels of staining for KDEL-containing proteins (Fig. 6) that were only observed at higher magnifications. As a second approach, we examined sections of diseased human colon for evidence of ER stress and HA deposition in vivo. In inflamed colon, numerous mucosal cells stained very intensely for KDEL-containing proteins, whereas tissue from untreated mice had only minimal staining for HA, and low levels of staining for KDEL-containing proteins in areas rich in HA. Likewise, regions with substantially less HA stained less strongly for KDEL-containing proteins (Fig. 7). These findings in both mouse and human tissue confirm that ER stress is associated with HA deposition in vivo.
FIG. 4. The effect of dextran sulfate is prolonged, not readily down-regulated, and does not require de novo protein synthesis. a, aortic SMCs were treated once with 10 μg/ml dextran sulfate for 23 h. Their ability to bind leukocytes was assayed, and replicate cultures were grown for an additional 4 days without dextran sulfate. Leukocyte adhesion was assayed every 24 h. ■ —■, control; ● —●, dextran sulfate. Data represent the mean ± S.D. n = 6; *, p < 0.05. b, aortic SMCs were treated continuously with 10 μg/ml dextran sulfate, and leukocyte adhesion was quantified daily. ■ —■, control; ● —●, dextran sulfate; ● —●, dextran sulfate and hyaluronidase. n = 6; *, p < 0.05. c, aortic SMCs were pretreated with 25 μg/ml cycloheximide for 1.5 h and then with or without 10 μg/ml dextran sulfate (Dex) and 25 μg/ml cycloheximide (CHX) for an additional 12 h. Leukocyte adhesion was quantified as described in the legend to Fig. 1. n = 6; *, p < 0.05.
DISCUSSION

Leukocyte extravasation from the circulation at sites of inflammation is regulated by the expression of specific adhesion molecules on the surface of both leukocytes and vascular endothelial cells. A number of investigations have demonstrated that activated leukocytes can bind to endothelial HA via the cell surface receptor CD44 both in vitro and in vivo (11). For instance, the adhesion of activated T cells to endothelium and their subsequent extravasation are mediated by endothelial HA and the HA-binding form of CD44 expressed by the lymphocytes (8–12). Deposition of HA on the surface of microvascular endothelial cells can be up-regulated by proinflammatory cytokines such as tumor necrosis factor-α, interleukin-1β, and interleukin-15 (11, 24). However, it is less clear what regulates the migration and adhesion of leukocytes once they enter the mesenchymal, connective tissue portions of inflamed tissue.

HA accumulates in numerous inflammatory conditions including inflammatory bowel disease, atherosclerosis, asthma, pancreatitis, and cirrhosis (6, 7, 14, 25–27). The accumulation of HA in areas of inflammation suggests that the production of HA by mesenchymal cells, including SMCs, may play an active role in regulating leukocyte adhesion and retention. The fact that HA-receptor interactions are associated with the differentiation of monocytes, with the activation of macrophages, and with leukocyte migration suggests that HA can have a dynamic role in the inflammatory process (28–30). However, little is known regarding factors that modulate the production of HA in inflamed tissue.

We have previously established that viral infection of colonic mucosal SMCs with live virus or with a viral product analogue, double-stranded RNA, dramatically up-regulates the binding of nonactivated leukocytes to mucosal SMCs (13). Two mechanisms of binding occur, a minor one mediated by vascular cell adhesion molecule-1 and a prominent, novel mechanism mediated by HA (13, 14). This latter mechanism is quite distinct from the tumor necrosis factor-α-induced up-regulation of cell adhesion molecules. Thus, whereas viral infection can induce the deposition of a leukocyte-binding form of HA, other factors that regulate the deposition and accumulation of this form of HA and the precise mechanisms of their actions are unknown.

A variety of environmental, metabolic, and pathological conditions induce cellular stress. The responses of cells to perturbations, such as ER stress, oxidative stress, and heat shock, serve to restore homeostasis. A variety of seemingly diverse factors may cause ER stress including inhibition of glycosylation, calcium depletion, viral infection, hyperhomocysteinemia, amino acid deprivation, hypoglycemia, cholesterol starvation, hypoxia, and the accumulation of misfolded proteins. Homeostasis in the ER is maintained by sensing mechanisms that detect unfolded, misfolded, or accumulating proteins (i.e. stress) in the ER lumen and by responding to stress with a series of complex, carefully orchestrated signaling events (16, 18, 23). In an effort to restore homeostasis, cells with ER stress respond by increasing the expression of numerous protective proteins involved in protein folding and protein degradation and by globally inhibiting protein synthesis to reduce the work load of the ER.

This is the first report to demonstrate directly that ER stress of SMCs induces leukocyte adhesion and that the adhesion occurs through an HA-mediated mechanism. Our results demonstrate that aortic SMCs were treated with 10 μg/ml dextran sulfate for 1, 2, 4, or 24 h, and then U937 cells were added for 1 h at 4 °C. The nonadherent cells were removed, and the cell layers were fixed and stained for HA (green) and nuclei (blue). The arrows point to HA-containing cables with bound leukocytes. Original magnifications were ×63 for the 1- and 2-h treatments and ×20 for the 4- and 24-h treatments.
onstrate that ER stress, regardless of its underlying cause, promotes the adhesion of leukocytes to SMCs. The bound leukocytes are removed by hyaluronidase treatment, demonstrating that HA-containing structures are important in the binding. Likewise, the binding of leukocytes to SMCs is prevented by treating the cell layer with hyaluronidase before the addition of leukocytes. Staining the cell layers for HA with affinity histochemistry showed that the HA is deposited extracellularly in stressed cultures and that it accumulates in a fibrillar, cable-like structure. Some of these cables also contain versican and Iα1, which are both known to bind to HA and which we have previously shown can colocalize with HA in cable structures (14, 31). HA staining also directly demonstrated the interaction of bound leukocytes with HA-containing structures.

Enhanced deposition of HA in stressed cultures is apparent within 1 h of treatment, well within the time period necessary to induce ER stress. The small strands of HA that are deposited in this short time are capable of binding leukocytes. Over time, the small strands appear to coalesce into larger thicker cables that also contain versican and Iα1. Together, the large number of small strands that are present after only 1 h are able to bind

3 A. Majors, unpublished observation.
40% as many leukocytes as do cultures treated for 23 h that contain large, thick cables. ER stress can thus rapidly induce both HA deposition by SMCs and leukocyte adhesion to SMCs.

SMCs treated with dextran sulfate for 24 h retained their capacity to bind leukocytes significantly above control levels for an additional 72 h. In the continued presence of dextran sulfate, SMCs were adhesive for exogenously added leukocytes for 5 days. These observations indicate that the deposited HA can persist for several days and that the cell’s ability to respond to stress and induce HA is not readily down-regulated. Together, these data are consistent with ER stress playing an active role in chronic inflammation.

Inhibition of protein synthesis by cycloheximide did not abrogate the ability of SMCs to deposit HA structures capable of mediating leukocyte adhesion. These results demonstrate that de novo synthesis of hyaluronan synthase(s) is not required and indicate that enhanced activity of the endogenous synthase(s) and/or inhibition of HA degradation may be responsible. Since HA is a glycosaminoglycan without any proteinaceous components, it can readily be made in the absence of protein synthesis, assuming that hyaluronan synthesis and its required substrates are available. Interestingly, cycloheximide by itself also induced HA deposition and leukocyte adhesion. This suggests that when cells are stressed by inhibiting protein synthesis, the production of HA is up-regulated, and it is deposited in a form that binds leukocytes. Other cellular stresses, however, such as heat shock and oxidant stress, do not elicit HA-mediated leukocyte binding to SMCs. The substantial accumulation of HA under unfavorable conditions such as ER stress, inhibition of protein synthesis, and viral infection is consistent with our previous report that HA synthase(s) is extraordinarily stable to cellular perturbations (32).

This in vitro model indicates that cells with either ER stress or inhibition of protein synthesis deposit excess HA into the extracellular matrix. To confirm this finding in vivo, we examined tissue sections from dextran sulfate-treated mice and also from surgical specimens of human colon to determine whether regions containing high levels of KDEL-containing proteins (ER-resident proteins), indicative of ER stress, are associated with enhanced staining for HA. Immunohistochemistry for KDEL-containing proteins and affinity histochemistry for HA demonstrated KDEL-containing proteins were abundantly expressed in numerous cells in regions rich in HA in inflamed colon from both the dextran sulfate-treated mice and from patients with inflammatory bowel disease. These findings indicate that HA deposition is associated with ER stress in vitro during inflammation.

Under seemingly disparate conditions such as ER stress, cycloheximide treatment, viral infection, and exposure to an analogue of the viral product double-stranded RNA, cells respond by producing a unique form of HA that binds leukocytes. A shared feature of all of these conditions is their global inhibition of protein synthesis. ER stress results in inhibition of eukaryotic translation initiation factor-2α (18, 23); cycloheximide inhibits peptidyl transferase (33); and viral infection or double-stranded RNA treatment strongly induces PKR, an inhibitor of eukaryotic translation initiation factor-3 (34), as well as double-stranded RNA-activated protein kinase (PKR), which inhibits eukaryotic translation initiation factor-2α (35). Under such conditions, where protein synthesis is attenuated, a non-protein moiety would be a preferred “signal” to label cells as “stressed.” We propose that an HA synthase complex with appropriate ancillary proteins serves as an alarm system that cells can utilize under conditions that hinder protein synthesis. The HA that is synthesized is thus organized in structures that serve as a distress signal and promote interactions with leukocytes.

Protein misfolding as a consequence of specific mutations can lead to protein retention in the ER and ER stress (15, 16, 36). For proteins that are abundantly expressed, such as many of those of the extracellular matrix, the ER may be severely affected. Therefore, our findings may be relevant to a number of heritable disorders of connective tissue. Mutations in type I collagen, the most abundant protein in the body, cause most forms of osteogenesis imperfecta (37). Specific mutations that are in the C terminus of the protein and that interfere with the association of the procollagen subunits, lead to the accumulation of procollagen chains in the ER and to ER stress and cause the most severe form of osteogenesis imperfecta (37–40). Interestingly, osteoblasts and fibroblasts from patients with osteogenesis imperfecta have been shown to deposit more HA than normal, control cells (41–43). Further studies are needed to determine whether the HA synthesized in this case is in the form that binds leukocytes and whether ER stress is responsible for this effect. Marfan syndrome is another connective tissue disorder where our observations may be noteworthy. A hallmark of Marfan syndrome is aortic aneurysms (44). Inflammation plays a significant role in the development of these aneurysms (45), and, interestingly, “ground substance,” now known to contain HA, accumulates in the aortas from patients with Marfan syndrome (44). Perhaps ER stress due to the accumulation of misfolded fibrillin-1 in the ER plays a role in the accumulation of “ground substance” and in the aortic inflammation in patients with mutations that hinder fibrillin-1 folding. Cystathionine β-synthase deficiency is a secondary disorder of connective tissue (46). The primary defect results in very high plasma levels of homocysteine, an amino acid known to cause ER stress both in vitro and in vivo (47–49). Consistent with our observations, arteries from patients with this disorder have an accumulation of “ground substance” (46). They also have premature atherosclerosis (i.e. chronic inflammation) (46). ER stress may also play a role in the pathogenesis of the vascular form of Ehlers Danlos syndrome type (IV). This disorder of type III collagen is characterized by distended ER in cells actively synthesizing type III collagen, aortic aneurysms, and intestinal ruptures (50). Thus, ER stress may play a role in the accumulation of HA and in the chronic inflammation found in various connective tissue diseases, and further studies on this possibility are warranted.

In summary, our data support a novel mechanism by which ER stress promotes the interaction of nonimmune and immune cells by enhancing the accumulation of a unique form of hyaluronan. Unlike other mechanisms, this interaction of SMCs and leukocytes is not mediated by cytokines but rather by metabolic, genetic, environmental, and infectious agents. Together with previous studies, our results suggest that the interactions between extracellular matrix and leukocytes represent a potential therapeutic target.

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