Role of Endoplasmic Reticulum Stress in Rheumatoid Arthritis Pathogenesis

Yune-Jung Park, Seung-Ah Yoo, and Wan-Uk Kim

INTRODUCTION

Rheumatoid arthritis (RA) is characterized by a tumor-like expansion of the synovium, which is composed of proliferating synoviocytes and infiltrating leukocytes, including T cells and B cells; these are likely activated by autoantigens (1). In RA joints, various inflammatory cells, including innate immune cells (e.g., mast cells, macrophages, dendritic cells [DCs], and natural killer cells), adaptive immune cells (T- and B cells), endothelial cells, and fibroblast-like synoviocytes (FLS), are activated (1-5). In particular, interleukin (IL)-17 producing T cells (the so-called T$_\text{H}$_17 cells) have emerged as one type of immune cell that is associated with the initiation and perpetuation of RA (6), and the modulation of IL-17 has been demonstrated to be effective for suppressing arthritis (6). These innate and adaptive immune cells interact via an array of cytokines and/or cell-to-cell contacts, which can also activate each other, leading to secretion of diverse cytochemokines, growth factors, and reactive oxygen species, which ultimately constructs persistent pro-inflammatory cascades (1-6).

The endoplasmic reticulum (ER) is the site of biosynthesis for all secreted and membrane proteins (7). The lumen of the ER is a unique environment, critical for proper folding of proteins destined for secretion or display on the cell surface (7). Homeostasis in the ER is maintained by a coordinated adaptive program, unfolded protein response (UPR) and ER-associated degradation (ERAD) (8-11). However, a variety of disturbances, including mutations that predispose proteins to misfolding in both substrate and pathway chaperones, altered cellular metabolism, and infection, can increase protein misfolding (7, 8). The accumulation of unfolded proteins in the ER leads to a condition known as ER stress. Failure of the ER's adaptive capacity results in abnormal activation of the unfolded protein response. Recently, we have demonstrated that ER stress-associated gene signatures are highly expressed in RA synovium and synovial cells. Mice with Grp78 haploinsufficiency exhibit the suppression of experimentally induced arthritis, suggesting that the ER chaperone GRP78 is crucial for RA pathogenesis. Moreover, increasing evidence has suggested that GRP78 participates in antibody generation, T cell proliferation, and pro-inflammatory cytokine production, and is therefore one of the potential therapeutic targets for RA. In this review, we discuss the putative, pathophysiological roles of ER stress and GRP78 in RA pathogenesis.

Keywords: Endoplasmic Reticulum Stress; GRP78/BiP; Pathogenesis; Arthritis; Rheumatoid
The ER stress response has also been recognized in a wide range of diseases, including cancer, hypoxia, ischemia/reperfusion injury, heart disease, neurodegenerative disorders, inflammatory bowel disease, obstructive airway disease, diabetes, and infection (7, 10, 11, 15-17). In particular, defects in the ER stress response have been implicated in chronic autoimmune inflammatory diseases (8, 10). Microarray analysis using the muscle tissue of patients with myositis has revealed that the expression of GRP78 is increased (18), suggesting that the ER response is involved in skeletal muscle damage in autoimmune myositis. Additionally, GRP78 is a target of auto-reactive B and T cell responses in a murine model of anti-Ro (SS-A) autoimmunity (19). Moreover, misfolded human leukocyte antigen-B27 (HLA-B27) has been suggested to promote spondyloarthropathy through abnormal ER stress responses (20-25). During assembly with β2m and the peptide in ER, HLA-B27 heavy chain has a tendency to misfold and to form aberrant disulfide-linked dimers (24). Enhanced accumulation of misfolded heavy chains can activate the abnormal UPR, and induce pro-inflammatory responses in spondyloarthopathies (25).

Collectively, earlier studies (8-10, 14, 19-21, 23, 25) have suggested that there is cross-talk between the ER stress response and chronic autoimmune inflammation, and that ER stress can induce or modify the phenotype of inflammatory diseases. However, the role of the ER stress response in the pathogenesis of RA remains to be defined. In fact, diverse stressful conditions, including hypoxia, low glucose, and the pro-inflammatory cytokine milieu, are frequently observed in the RA joints (26), and these might act as ER stressors. In this review, we integrate the current knowledge on the possible link between the ER stress response and RA, a representative chronic inflammatory disease, focusing on the role of GRP78, and propose potential pathophysiological effects and therapeutic implications of GRP78 in RA pathogenesis.

**GRP78 Regulation of Fibroblast-Like Synoviocytes (FLS) Proliferation**

In various tumor models, GRP78, a central regulator of the ER stress response, plays an important role in resisting stressful host microenvironments and facilitating cell survival (27-33). For example, hypoxia-induced ER stress causes enhanced GRP78 expression, which increases cellular survival and adaptation to the microenvironment in colorectal cancer (28). Surface GRP78 on prostate cancer cells can bind activated α2-M and promote cellular proliferation and survival by activation of extracellular signal-regulated kinases (ERK) 1/2, p38 mitogen-activated protein kinases, phosphatidylinositol 3-kinases/protein kinase B, and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling cascades, as well as by elevation of the GRP78 expression itself (29-31). ER transmembrane GRP78 forms a complex with caspase-7 or caspase-12, and inhibits caspase-7 activation-induced cell death (32). GRP78 also co-localizes with Raf-1 on the outer membrane of mitochondria to maintain mitochondrial permeability and thus protect cells from ER stress-induced apoptosis (33).

The pathologic hallmark of RA is the “invasive pannus” that results from synovioyte hyperplasia (1-4). Rheumatoid synoviocytes, which consist of FLS and synovial macrophages, exhibit invasive characteristics reminiscent of cancer cells, destroying cartilage and bone. They are responsible for many aspects of RA pathology, such as synovial proliferation, perpetuation of chronic inflammation, and joint destruction (34-36). In the inflamed RA synovium, it was demonstrated that the FLS of RA patients (RA-FLS) exhibit considerable morphologic alterations (34-37). They have abundant cytoplasm, a dense rough ER, and large pale nuclei. It has been postulated that chronic exposure of FLS to a combination of inflammatory cytokines, growth factors, and chronic hypoxia results in continued activation of FLS, exhibiting some features of tumor cells, such as anchorage-independent growth, alterations in their response to apoptotic stimuli, and migration/invasion toward articular cartilage and bone. Moreover, as seen in Fig. 1, RA-FLS were more resistant to apoptosis induced by ER stressors (e.g., tunicamycin and thapsigargin) than FLS of osteoarthritis (OA) patients. These features of RA-FLS are referred to as “tumor-like” transformation or dedifferentiation (36, 37). However, it remains unclear how RA-FLS exhibit a transformed phenotype.

Recently, we have provided a glimpse of evidence on how RA-FLS exhibit abnormal proliferation in inflamed joints. We were the first to demonstrate that ER stress–associated gene signatures, induced by chronic hypoxia and pro-inflammatory cytokines, are responsible for the abnormal proliferation of RA-FLS and angiogenesis, namely “pannus formation” (38). Down-regulation of GRP78, a master regulator of the ER stress response, increases apoptosis of RA-FLS. Conversely, overexpression of GRP78 prevents RA-FLS from apoptotic death induced by an ER stressor (38). Moreover, GRP78 controls synoviocyte proliferation and angiogenesis in vivo (38). Mice with Grp78 haplo-insufficiency exhibit the suppression of experimentally induced arthritis, and develop a limited degree of synovial proliferation and angiogenesis (38). We suggest that the ER chaperone GRP78 is critical for synoviocyte proliferation and angiogenesis, the pathologic hallmark of RA, and may be responsible for FLS transformation. In conclusion, our findings provide new insights into the role of GRP78 in the pathogenesis of RA, and explain how normal synoviocytes develop an aggressive phenotype in RA joints.
ROLE OF ER STRESS RESPONSE IN LYMPHOCYTE ACTIVATION

**GRP78 and B cell activation**

B cells are directly or indirectly involved in the pathogenesis of RA. In RA joints, they can differentiate into plasma cells. The infiltrating plasma cells in rheumatoid synovium synthesize the pathogenic autoantibodies, such as immunoglobulin M rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibodies (ACPA) (39, 40). These autoantibodies are crucial to the initiation and perpetuation of chronic inflammation, and their presence in the sera have been widely utilized as diagnostic and prognostic markers of RA (41, 42). In recent years, clinical benefits of B cell ablation therapy (e.g., rituximab treatment) have confirmed the important role of B cells in the propagation of RA inflammation (41). Differentiation of B cells into plasma cells in response to antigenic stimuli usually requires a massive increase in the biosynthetic capacity to produce the autoantibodies within the ER (43-45). Thus, the ER stress response seems to play a role in the development of the immunoglobulin-secreting plasma cells (43, 44). In support of this, activation of the UPR promotes the expression of GRP78 in plasma cells (45-47), and the increased GRP78 expression represents an important pro-survival component of the secretory cells, including antibody-secreting plasma cells (48-50).

Abnormal antibody responses to GRP78 have been associated with the pathogenesis of RA. Serum anti-GRP78 antibody is detected in up to 63% of RA patients (51), indicating that rheumatoid B cells recognize GRP78 as an autoantigen; however, downstream effect of the anti-GRP78 antibody-GRP78 complex remains unclear. A recent study has demonstrated that the expressions of GRP78 is more intensive in infiltrating plasma cells in RA synovium than in OA synovium; a positive relationship between the expression of GRP78 in plasma cells from synovial fluid and ACPA levels is found in RA (45). In addition, antibodies to citrullinated GRP78 are also frequently found in RA patients (52), suggesting that citrullinated GRP78 is one of the ACPA targets. Moreover, immunization of mice with citrullinated GRP78 induces several kinds of ACPA (52), which suggests that citrullinated GRP78 contributes to chronic arthritis via generation of...
ACPA. Taken together, generation of pathogenic autoantibodies to GRP78 and/or citrullinated GRP78, in addition to enhanced activation of the UPR in infiltrating plasma cells, occurs in the rheumatoid synovium, and thus they may contribute to autoimmune arthritis.

**GRP78 effect on T cell activation and application to treatment**

Antigen peptides presented in the HLA-DR groove activate CD4+ T cells (53). A strong association between disease susceptibility and specific major histocompatibility complex (MHC) class II molecules in RA indicates that CD4+ T cells may be involved in disease development. In fact, autoantigen-triggered T cells, particularly Tn11 and Tn17 cells, have been thought to play an important role in the progression of autoimmune polyarthritis, including RA. For example, immunization of susceptible strains of mice with type II collagen (CII), one of the cartilage components (autoantigens), leads to the development of an autoimmune polyarthritis by inducing Tn1 and Tn17 cells to respond to CII (54). CII-reactive CD4+ T cell lines have been reported to transfer disease to naive mice (55). Moreover, numbers of CII-reactive T cells are increased in RA patients and are associated with a shift to Tn1 cytokine production (56), indicating that they may be capable of initiating or perpetuating RA.

Evidence is emerging that aberrant UPR in T cells contributes to the development of chronic arthritis. MHC antigen presentation is fundamentally connected to the ER because peptides for loading onto MHC are generated from both cytosolic and ER-derived proteins (8). During ERAD, misfolded proteins accumulated within ER can lead to greater presentation on MHC at the cell surface, resulting in an increased chance of activation of autoreactive T cells (8). In addition, several studies suggest that GRP78 is a major autoantigenic target for the T cells of RA patients (51). T cell proliferation assays indicate that GRP78-specific T cells are found in 68% of RA patients. They also can proliferate, despite the presence of large amounts of the suppressive cytokine IL-10 (51). Therefore, GRP78 is recognized as a self-antigen by RA T cells as well as RA B cells.

Single high-dose or repetitive low-dose administration of self-antigens is a well-established procedure for inducing peripheral immune tolerance, which suppresses autoimmune responses and disease severity in animal models of experimental allergic encephalomyelitis, collagen-induced arthritis (CIA), experimental uveitis, and non-obese diabetes (57-60). For example, antigen-specific T cell suppression using low-dose CII ameliorates arthritis in animal models and disease activity in some RA patients (59); both are also mediated by active induction of immune-suppressive cytokines, such as IL-4, IL-10, and transforming growth factor (TGF)-β (59). Similarly, it can be expected that treatment of exogenous GRP78 may suppress arthritis severity because GRP78 is a specific T cell antigen for RA (51). Corrigall et al. have demonstrated that administration of extracellular GRP78 suppresses active CIA by the induction of regulatory cells that act predominantly via IL-4 (51). Moreover, the addition of extracellular GRP78 to normal peripheral blood mononuclear cells (PBMCs) stimulates immune-modulatory and anti-inflammatory pathways, which are partly due to the production of IL-10 in PBMCs (61). Thus, exogenous, extracellular GRP78 might have an immuno-suppressive function in RA patients. Interestingly, exogenous heat shock protein (HSP), another molecular chaperone involved in RA pathogenesis, suppresses autoimmune T cell responses and arthritis severity in mice (62), indicating that chaperone-induced tolerance induction is not restricted to GRP78. In fact, regulatory T cells that recognize a ubiquitous stress-inducible self-antigen, such as HSP70, are long-lived suppressors of autoimmune arthritis (63). Given the high level of expression of ER stress proteins in RA synovium (38), other ER response-associated molecules, such as recombinant ATF6 and IRE1, can be tested for their potential as tolerance-inducing agents to suppress chronic arthritis.

GRP78 is constitutively expressed in B cells or T cells (64, 65). Activation of T cell receptors (TCRs) induces ER stress-associated UPR including chaperone proteins (66, 67). GRP78 is induced by TCR-mediated signaling via a Ca2+ dependent pathway and plays a critical role in maintaining T cell viability in the steady and TCR-activated states (66). GRP78 expression is also increased in T cells stimulated with phorbol 12-myristate 13-acetate (67). This process might be regulated by protein kinase C-signaling pathways (67). In a recent study, GRP78 deficiency was shown to attenuate granzyme B-mediated cytotoxicity and to reduce T cell proliferation in CD8α+ T cells (68), suggesting that GRP78 regulates T cell function. However, it remains largely unknown how essential GRP78 is for their activation, differentiation, proliferation, and survival in CD4+ T cells. Thus, it would be informative to test whether intracellular GRP78 is necessary for the pathophysiology of CD4+ cells and for the development of T cell-dependent autoimmune diseases, such as RA.

**GRP78 AND PRO-INFLAMMATORY CYTOKINE PRODUCTION**

Accumulating evidence suggests that ER stress is involved in the proinflammatory process (9). For example, the pro-inflammatory cytokines, including IL-1β and tumor necrosis factor alpha (TNF-α), have been reported to induce the ER stress response in hepatocytes, leading to the activation of CREBH, a transcription factor that stimulates the expression of proteins involved in the acute inflammatory response, such as serum amyloid P-component and C-reactive protein (69). In murine fibrosarcoma cells, TNF-α was found to trigger UPR, increasing the expressions of XBP1 and GRP78 (70). Selective abrogation of GRP78 by subtilase cytotoxin blunts activation of the pro-inflammatory cytokine TNF-α, indicating that the expression of GRP78 is dependent on the presence of an intact UPR. The importance of GRP78 in maintaining T cell viability and function is further supported by the finding that GRP78 regulates T cell responses in vivo, as evidenced by the increased susceptibility of GRP78-deficient mice to experimental autoimmune encephalomyelitis (71). Therefore, it is likely that the expression of GRP78 in T cells plays a critical role in maintaining T cell viability and function, which is essential for the development of T cell-mediated autoimmune diseases, such as RA.
Park Y-J, et al. • ER Stress and Rheumatoid Arthritis

Inflammatory NF-κB signal pathway, and protects mice from endotoxic lethality and CIA (71). We have also demonstrated that pro-inflammatory cytokines can induce GRP78 expression in RA-FLS (38). Together, the previous findings (9, 38, 69-71) indicate a link between ER stress and inflammation, suggesting that ER stress is one of the major mediators of chronic inflammation.

GRP78 expression is not limited to the ER, but is significantly identified on cell surface (72). As seen in Fig. 2, we identified that FLS expressed GRP78 on the cell surface in addition to ER, and that surface GRP78 levels were higher in RA-FLS than in OA-FLS. Interestingly, a recent report has shown that citrullinated GRP78 on the surface of monocyte/macrophage acts as a receptor for ACPA to enhance activation of the inflammatory NF-κB pathway and production of inflammatory cytokine TNF-α (65). Therefore, it is possible that ACPA may bind to GRP78 on RA-FLS or RA synovial macrophages, and then trigger cytokeratin production by inducing NF-κB. The resultant increase in pro-inflammatory cytokines may further induce GRP78 expression in RA-FLS and FLS proliferation (38), constructing a feed-forward cycle of rheumatoid inflammation. If this is the case, therapeutic agents targeting surface GRP78 can be effective for the selective incapacitation of invasive RA-FLS, as they were for some types of cancer (27).

An endogenous intracellular chaperone molecule, released

Fig. 2. GRP78 is expressed in the ER and membrane of synoviocytes. (A and B). Immuno-fluorescence staining of GRP78 in FLS. RA synoviocytes were permeabilized, and stained with anti-GRP78 antibody and CellLight ER-RFP, an ER marker. Images were obtained by confocal microscopy. (a) phase contrast image, colocalization of GRP78 (b, green) with ER marker (c, red) is shown in orange (d, merge). Scale bars: 100 µm. (C and D). FACS analysis of synoviocytes obtained from OA (C) or RA patients (D). Cells were stained with DyLight 488-conjugated anti-GRP78 antibody, and were analyzed by flow cytometry. Red histograms correspond to specific labeling for surface GRP78 and gray histograms indicate isotypic control antibody. GRP78, glucose-regulated protein of 78 kDa; FLS, fibroblast-like synoviocytes; RA, rheumatoid arthritis; ER, endoplasmic reticulum; FACS, fluorescence-activated cell sorting; OA, osteoarthritis.
at times of acute or chronic physiological stress as a form of exosome, necrotic or apoptotic debris, can contribute to immunomodulating signals within the immune network through a variety of mechanisms (73). As reported previously (61), we found that cell-free GRP78 was frequently found in the synovial fluid of RA patients (Fig. 3A). Interestingly, when GRP78 was added to synovial mononuclear cells of patients with RA, the production of IL-17 and TNF-α was increased (Fig. 3B). Consistent with Corrill et al’s study (61), such an increase was not noted with OA or normal PBMCs (Fig. 3B). In addition, exogenous GRP78 increased IL-23 production by lipopolysaccharide-stimulated DCs, while simultaneously decreasing IL-10 production by these cells (Fig. 3C), indicating that the GRP78-induced increase in IL-17 production was mediated by the modulation of IL-10 and IL-23 production from mature DCs. Moreover, GRP78 treatment to immature DCs unregulated the expression of co-stimulatory molecules, such as CD40 and CD80. The CD40 and CD86 expressions in DCs stimulated with TNF-α were also additively increased by the treatment with GRP78 (Fig. 3D). These data provide additional evidence for the GRP78-induced increase in chronic inflammatory responses in RA.

Taken together, pro-inflammatory cytokines up-regulate GRP78 expression in RA-FLS. The increased GRP78 expression, in turn, could further activate RA-FLS by interacting with ACPA as a surface form and as a soluble form by triggering IL-17 production and co-stimulatory molecule expression in RA synovial mononuclear cells.

**GRP78-MEDIATED ANGIGENESIS**

GRP78 is induced in hypoxic endothelial cells (74), and is up-regulated by vascular endothelial growth factor (VEGF) treatment (75). GRP78 knockdown significantly suppresses VEGF-induced activation of ERK1/2, phosphoinositide phospholipase C, and VEGF receptor-2 (VEGFR-2) as well as VEGF-induced endothelial cell proliferation (75). Several lines of evidence have shown that GRP78 promotes tumor angiogenesis. Kringle 5 (K5) of human plasminogen can function as a binding partner of GRP78 on the cell surface of proliferating endothelial cells (74). Conditional knockout mice of GRP78 in the endothelial cells

---

**Fig. 3.** Recombinant GRP78 induces pro-inflammatory response in rheumatoid mononuclear cells. (A) Expression of GRP78 in the synovial fluid of RA patients (n = 8), which was determined by Western blot analysis. (B) GRP78-induced production of IL-17 and TNF-α by synovial fluid mononuclear cells of RA patients (n = 3) versus peripheral blood mononuclear cells of OA (n = 3). Mononuclear cells (1 × 10⁶) were stimulated with recombinant GRP78 for the indicated time. Cytokine concentrations in the culture supernatants were determined by ELISA. Data are the mean ± SD, and are presented as the fold increase as compared with media only. (C) Increase in IL-10 and IL-23 production by recombinant GRP78. RA mononuclear cells (1 × 10⁶) were stimulated with recombinant GRP78 in the presence of LPS (1 µg/mL) for 24 hr. The IL-10 and IL-23 levels in the culture supernatants were determined by ELISA. (D) GRP78-induced upregulation of co-stimulatory molecules on dendritic cells (DCs). RA mononuclear cells (1 × 10⁶) were stimulated with recombinant GRP78 (10 ng/mL) for 24 hr, and the expressions of CD40, CD80, and CD86 on immature DCs were analyzed by flow cytometry. RA, rheumatoid arthritis; GRP78, recombinant glucose-regulated protein of 78; IL, interleukin; OA, osteoarthritis; TNF-α, tumor necrosis-factor alpha; ELISA, enzyme-linked immunosorbent assay; SD, standard deviation; LPS, lipopolysaccharide.
can cause dramatic reduction of tumor angiogenesis (76). Knockdown of GRP78 expression in human endothelial cells reduces angiogenesis by suppressing cell proliferation, survival, and migration (76). It has also been demonstrated that cell-surface GRP78-targeting peptide has an anti-angiogenic effect (77, 78). The bacterial AB5 subtilase cytotoxin can specifically cleave GRP78 at a single amino acid, abolishing GRP78 function rapidly and specifically (77). Conjugation of GRP78 with the plasminogen K5 or extracellular Par-4 promotes endothelial apoptosis, which suggests that cell-surface GRP78-targeting peptide can be utilized as a potential anti-angiogenesis therapy.

Angiogenesis is highly active in RA, particularly in the early onset of the disease (3, 4). The newly formed vessels can maintain the chronic inflammatory state by transporting the inflammatory cells to the site of synovitis, as well as supplying nutrients and oxygen to the synovium (3, 4). Of many angiogenic factors, VEGF plays a central role in “pannus formation” (3, 4). In RA, VEGF appears in increased amounts in the sera, synovial fluids, and inflamed synovium of patients (79), and thus constitutes a potential candidate for therapeutical modulation. Treatment with anti-VEGF antibody has been shown to attenuate CIA in mice (80). Again, specific inhibition of VEGF by soluble VEGF receptors reduced the disease severity in murine CIA (80). Our group has shown that GRP78 deficiency inhibits VEGF165 stimulated endothelial cell proliferation (38). In addition, VEGF-induced tube formation, migration, and chemotaxis of endothelial cells are also markedly reduced by knockdown of GRP78. These results, together with previous reports (38, 79, 80), indicate that GRP78 directly mediates VEGFα-induced migration, chemotaxis, and endothelial cell proliferation. Thus, anti-GRP78 inhibitors could be effective for suppressing the excessive angiogenesis frequently noted in RA joints.

ASSOCIATION OF ASSOCIATED DEGRADATION (ERAD) WITH UNFOLDED PROTEIN RESPONSE (UPR) IN RA

In addition to UPR, ERAD is also required to avoid ER stress in the cells (8-11). The UPR relieves ER stress by inducing ER chaperones to increase the protein-folding capacity of the ER, as well as by inhibiting general protein translation. In contrast, the ERAD eliminates misfolded or unassembled proteins that accumulate in the ER through the ubiquitin–proteasome system (8-11). Unless two compensatory mechanisms of UPR and ERAD work properly, ER stress causes cell damage, and eventually cell death (8-11). Synoviolin is one of the ER-resident E3 ubiquitin ligases involved in ERAD, and is implicated in RA pathogenesis (81, 82). Several studies have shown the relationship between GRP78 and synoviolin (72, 83). In stressed cells, increased GRP78 expression is associated with activation of P58IPK and other chaperones, which enhances ERAD in the ER lumen (72). In zebrafish embryonic cell line ZF4, endogenous IGF1 is induced as XBP-1 splicing during ER stress, and XBP-1 not only increases GRP78 but also induces synoviolin (83). Such findings (72, 81-83) suggest that the ERAD system is closely related to UPR.

As mentioned above, RA-FLS are the major cell population in tumor-like expansion and invasive pannus. In the inflamed joints, RA synovial cells have to keep producing large amounts of proteins for the progression of inflammation. In this context, ERAD may be a necessary processing system for homeostasis (84). Indeed, ERAD is aberrantly unregulated in RA (85). A recent study has demonstrated that overexpression of synoviolin causes arthropathy with synovial hyperplasia, whereas knockdown of synoviolin results in increased apoptosis of synovial cells and less sensitivity to CIA in mice (85). Enhanced ERAD may efficiently remove unfolded protein in ER, which results in the indirect suppression of UPR activation (85). This notion is supported by previous findings that mouse embryonic fibroblasts that lack synoviolin show increased susceptibility to ER stress-induced apoptosis (81, 86). The previous reports on ERAD (85, 86) are consistent with our data in that dysregulated ER responses critically contribute to synovial hyperplasia and the development of chronic arthritis. Thus, it would be interesting to investigate whether two biological processes, UPR and ERAD, affect each other to induce RA.

CONCLUSION AND PERSPECTIVE

The possible role of ER stress in RA pathogenesis is summarized in Fig. 4. Micro-environmental stresses such as hypoxia, glucose deprivation, reactive oxygen species, and pro-inflammatory cytokines, may increase ER stress in both innate immune...
cells (e.g., DCs and FLS) and adaptive immune cells (e.g., T and B cells) in inflamed joints. In particular, during ER stress, GRP78 expression is increased in RA-FLS. The increased GRP78 expression promotes FLS survival and proliferation, resulting in synovial proliferation. The induction of GRP78 by ER stress may lead to an increase in GRP78 in the ER lumen as well as promotion of GRP78 re-localization from the ER to the cell surface; in this case, cell surface GRP78 can be a target for ACA and may act as an auto-antigen for T and B cells. Moreover, extracellular GRP78, detected at high levels in RA joints, may contribute to the development of auto-reactive T cells and increase the production of IL-17 and TNF-α in RA synovial mononuclear cells. In addition, citrullinated GRP78 on monocytes/macrophages binds to ACA, and stimulates the production of pro-inflammatory cytokines, such as TNF-α, which further increases GRP78 expression in RA-FLS. Increased GRP78 expression in RA-FLS, in turn, could amplify the inflammatory cascade by escalating pannus formation. Finally, GRP78 directly stimulates VEGF-induced migration/chemotaxis and endothelial cell proliferation, which facilitate synovial angiogenesis.

GRP78 is traditionally regarded as a major ER chaperone (7, 9, 10, 15). However, increasing evidence indicates that GRP78 exists outside the ER, in the cytoplasm and cell membrane, and plays a critical role in cell survival, tumor angiogenesis, metastasis, and resistance to cancer therapy (7, 8, 10, 27, 72). In this regard, our finding that GRP78 is present on the surface of FLS may open the door to novel therapeutic approaches that specifically target synovioyte proliferation and endothelial cells, the pathologic hallmark of RA. For example, conjugation of toxin- or apoptosis-inducing agents with synthetic peptides that can bind to GRP78, such as WIFPWQL (73), may inhibit synovial proliferation, angiogenesis, and the pannus formation. In addition, it can be expected that extracellular GRP78 could suppress RA activity by inducing T cell tolerance and also by competing with membrane GRP78 for binding of the anti-GRP78 antibody. We are currently investigating such possibilities.

**DISCLOSURE**

The authors declare no potential conflicts of interest.

**REFERENCES**

1. McInnes IB, Schett G. *The pathogenesis of rheumatoid arthritis*. N Engl J Med 2011; 365: 2205-19.
2. Firestein GS. *Invasive fibroblast-like synoviocytes in rheumatoid arthritis: passive responders or transformed aggressors?* Arthritis Rheum 1996; 39: 1781-90.
3. Firestein GS. Starving the synovium: angiogenesis and inflammation in rheumatoid arthritis. J Clin Invest 1999; 103: 3-4.
4. Koch AE. Review: angiogenesis: implications for rheumatoid arthritis.

Arthritis Rheum 1998; 41: 951-62.
5. Jung YO, Kim HA. Recent paradigm shifts in the diagnosis and treatment of rheumatoid arthritis. Korean J Intern Med 2012; 27: 378-87.
6. Maddur MS, Misoeec P, Kaveri SV, Bayry J. Th17 cells: biology, pathogenesis of autoimmune and inflammatory diseases, and therapeutic strategies. Am J Pathol 2012; 181: 8-18.
7. Xu C, Baillie-Maitre B, Reed JC. Endoplasmic reticulum stress: cell life and death decisions. J Clin Invest 2005; 115: 2656-64.
8. Hasnain SZ, Lourie R, Das I, Chen AC, McGuckin MA. The interplay between endoplasmic reticulum stress and inflammation. Immunol Cell Biol 2012; 90: 260-70.
9. Zhang K, Kaufman RJ. From endoplasmic-reticulum stress to the inflammatory response. Nature 2008; 454: 455-62.
10. Yoshida H. ER stress and diseases. FEBS J 2007; 274: 630-58.
11. Niedereiter L, Kaser A. Endoplasmic reticulum stress and inflammatory bowel disease. Acta Gastroenterol Belg 2011; 74: 330-3.
12. Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. Nat Rev Mol Cell Biol 2007; 8: 519-29.
13. Schröder M, Kaufman RJ. The mammalian unfolded protein response. Annu Rev Biochem 2005; 74: 739-89.
14. Todd DJ, Lee AH, Gilmerch LH. The endoplasmic reticulum stress response in immunity and autoimmunity. Nat Rev Immunol 2008; 8: 663-74.
15. Marcink SJ, Ron D. The unfolded protein response in lung disease. Proc Am Thorac Soc 2010; 7: 356-62.
16. Roussel BD, Krupka AJ, Miranda E, Crowther DC, Lomas DA, Marcink SJ. Endoplasmic reticulum dysfunction in neurological disease. Lancet Neurol 2013; 12: 105-18.
17. Kitamura M. Endoplasmic reticulum stress in the kidney. Clin Exp Nephrol 2008; 12: 317-25.
18. Nagaraju K, Casciola-Rosen L, Lindenberg I, Rawat R, Cutting S, Thapliyal R, Chang I, Dwivedi S, Mitsak M, Chen YW, et al. Activation of the endoplasmic reticulum stress response in autoimmune myositis: potential role in muscle fiber damage and dysfunction. Arthritis Rheum 2005; 52: 1824-35.
19. Gordon TP, Bolstad AI, Rischmueller M, Jonsson R, Waterman SA. Autoantibodies in primary Sjögren’s syndrome: new insights into mechanisms of autoantibody diversification and disease pathogenesis. Autoimmunity 2001; 34: 123-32.
20. Colbert RA, DeLay ML, Klenk EL, Layh-Schmitt G. From HLA-B27 to spondyloarthritis: a journey through the ER. Immunol Rev 2010; 233: 181-202.
21. DeLay ML, Turner MJ, Sowders DP, Colbert RA. HLA-B27 misfolding and the unfolded protein response augment interleukin-23 production and are associated with HLA-B27 activation in transgenic rats. Arthritis Rheum 2009; 60: 2633-43.
22. Turner MJ, Sowders DP, DeLay ML, Mohapatra R, Bai S, Smith JA, Brandwein JR, Taurog JD, Colbert RA. HLA-B27 misfolding in transgenic rats is associated with activation of the unfolded protein response. J Immunol 2005; 175: 2438-48.
23. Smith JA, Barnes MD, Hong D, DeLay ML, Inman RD, Colbert RA. Gene expression analysis of macrophages derived from ankylosing spondylitis patients reveals interferon-gamma dysregulation. Arthritis Rheum 2008; 58: 1640-9.
24. Dangoria NS, DeLay ML, Kingsbury DJ, Mear JP, Uchanska-Ziegler B, Ziegler A, Colbert RA. HLA-B27 misfolding is associated with aberrant

http://dx.doi.org/10.3346/jkms.2014.29.1.2

http://jkms.org
intermolecular disulfide bond formation (dimerization) in the endoplasmic reticulum. J Biol Chem 2002; 277: 23459-68.
25. Turner MJ, Delay ML, Bai S, Klenk E, Colbert RA. HLA-B27 up-regulation causes accumulation of misfolded heavy chains and correlates with the magnitude of the unfolded protein response in transgenic rats: implications for the pathogenesis of spondylarthritides-like disease. Arthritis Rheum 2007; 56: 215-23.
26. Stevens CR, Williams RB, Farrell AJ, Blake DR. Hypoxia and inflammatory synovitis: observations and speculation. Ann Rheum Dis 1991; 50: 124-32.
27. Li Z, Li Z. Glucose regulated protein 78: a critical link between tumor microenvironment and cancer hallmarks. Biochim Biophys Acta 2012; 1826: 13-22.
28. Verras M, Papandreou I, Lim AL, Denko NC. Tumor hypoxia blocks Wnt processing and secretion through the induction of endoplasmic reticulum stress. Mol Cell Biol 2008; 28: 7212-24.
29. Misra UK, Gonzalez-Gronow M, Gawdi G, Hart JP, Johnson CE, Pizzo SV. The role of Grp 78 in alpha 2-macroglobulin-induced signal transduction: evidence from RNA interference that the low density lipoprotein receptor-related protein is associated with, but not necessary for, GRP 78-mediated signal transduction. J Biol Chem 2002; 277: 42082-7.
30. Misra UK, Deedwania R, Pizzo SV. Activation and cross-talk between Akt, NF-kappaB, and unfolded protein response signaling in 1-LN prostate cancer cells: subsequent to ligation of cell surface-associated GRP78. J Biol Chem 2006; 281: 13694-707.
31. Misra UK, Wang F, Pizzo SV. Transcription factor TFII-I causes transcriptional upregulation of GRP78 synthesis in prostate cancer cells. J Cell Biochem 2009; 106: 381-9.
32. Reddy RK, Mao C, Baumeister P, Austin RC, Kaufman RJ, Lee AS. Endoplasmic reticulum chaperone proteinGRP78 protects cells from apoptosis induced by topoisomerase inhibitors: role of ATP binding site in suppression of caspase-7 activation. J Biol Chem 2003; 278: 20915-24.
33. Shu CW, Sun FC, Cho JH, Lin CC, Liu PF, Chen PY, Chang MD, Fu HW, Lai YK. GRP78 and Raf-1 cooperatively confer resistance to endoplasmic reticulum stress-induced apoptosis. J Cell Physiol 2008; 215: 627-35.
34. Buckley CD, Pilling D, Lord JM, Akbar AN, Scheel-Toellner D, Salmon M. Fibroblasts regulate the switch from acute resolving to chronic persistent inflammation. Trends Immunol 2001; 22: 199-204.
35. Qu Z, Garcia CH, O’Rourke LM, Planck SR, Kohli M, Rosenbaum JT. Local proliferation of fibroblast-like synoviocytes contributes to synovial hyperplasia: results of proliferating cell nuclear antigen/cyclin, c-myc, and nucleolar organizer region staining. Arthritis Rheum 1994; 37: 212-20.
36. Pap T, Miller-Ladner U, Gay RE, Gay S. Fibroblast biology. Role of synovial fibroblasts in the pathogenesis of rheumatoid arthritis. Arthritis Res 2000; 2: 361-7.
37. Fassbender HG. Histomorphological basis of articular cartilage destruction in rheumatoid arthritis. Coll Relat Res 1983; 3: 141-55.
38. Yoo SA, You S, Yoon HJ, Kim DH, Kim HS, Lee K, Ahn JH, Hwang D, Lee AS, Kim KJ, et al. A novel pathogenic role of the ER chaperone GRP78/ Bip in rheumatoid arthritis. J Exp Med 2012; 209: 871-86.
39. Masson-Bessière C, Sebbag M, Durieux JI, Nogueira L, Vincent C, Girbal-Neuhauser E, Durroux R, Cantagrel A, Serre G. In the rheumatoid pannus, anti-filaggrin autoantibodies are produced by local plasma cells and constitute a higher proportion of IgG than in synovial fluid and serum. Clin Exp Immunol 2000; 119: 544-52.
40. Kim HR. Anti-citrullinated protein antibodies in rheumatoid arthritis: a bridge between genetic predisposition and autoimmunity. Korean J Intern Med 2013; 28: 25-8.
41. Dörner T, Burmester GR. The role of B cells in rheumatoid arthritis: mechanisms and therapeutic targets. Curr Opin Rheumatol 2003; 15: 246-52.
42. Choi SW, Lim MK, Shin DH, Park JI, Shim SC. Diagnostic performances of anti-cyclic citrullinated peptides antibody and anti-filaggrin antibody in Korean patients with rheumatoid arthritis. J Korean Med Sci 2005; 20: 473-8.
43. Brewer JW, Hendershot LM. Building an antibody factory: a job for the unfolded protein response. Nat Immunol 2005; 6: 23-9.
44. Gass JN, Gunn KE, Sriburi R, Brewer JW. Stressed-out B cells? plasma cell differentiation and the unfolded protein response. Trends Immunol 2004; 25: 17-24.
45. Dong W, Li X, Feng Y, Fan C, Chen Z, Zhu P. The differential expressions of 78-kDa glucose-regulated protein of infiltrating plasma cells in peripheral joints with the histopathological variants of rheumatoid synovitis. Arthritis Res Ther 2009; 11: R4.
46. Bánhegyi G, Baumeister P, Benedetti A, Dong D, Fu Y, Lee AS, Li J, Mao C, Margittai E, Ni M, et al. Endoplasmic reticulum stress. Ann N Y Acad Sci 2007; 1113: 58-71.
47. Gass JN, Jiang HY, Wek RC, Brewer JW. The unfolded protein response of B-lymphocytes: PERK-independent development of antibody-secreting cells. Mol Immunol 2008; 45: 1035-43.
48. Lee AS. The glucose-regulated proteins: stress induction and clinical applications. Trends Biochem Sci 2001; 26: 504-10.
49. Bernales S, Papa FR, Walter P. Intracellular signaling by the unfolded protein response. Annu Rev Cell Dev Biol 2006; 22: 487-508.
50. Lee AS. The ER chaperone and signaling regulator GRP78/BIP as a monitor of endoplasmic reticulum stress. Methods 2005; 35: 373-81.
51. Corrigall VM, Bodman-Smith MD, Fife MS, Canas B, Myers LK, Wooley P, Soh C, Staines NA, Pappin DJ, Berlo SE, et al. The human endoplasmic reticulum molecular chaperone BiP is an autoantigen for rheumatoid arthritis and prevents the induction of experimental arthritis. J Immunol 2001; 166: 1492-8.
52. Shoda H, Fujio K, Shibuya M, Okamura T, Sumitomo S, Okamoto A, Sawada T, Yamamoto K. Detection of autoantibodies to citrullinated BiP in rheumatoid arthritis patients and pro-inflammatory role of citrullinated BiP in collagen-induced arthritis. Arthritis Res Ther 2011; 13: R191.
53. Panayi GS. T-cell-dependent pathways in rheumatoid arthritis. Curr Opin Rheumatol 1997; 9: 236-40.
54. Boissier MC, Feng XZ, Carlioz A, Roudier R, Fournier C. Experimental autoimmune arthritis in mice: I. homologous type II collagen is responsible for self-perpetuating chronic polyarthritis. Ann Rheum Dis 1987; 46: 691-700.
55. Kakimoto K, Katsuaki M, Hirofujii T, Iwata H, Koga T. Isolation of T cell line capable of protecting mice against collagen-induced arthritis. J Immunol 1988; 140: 78-83.
56. Park SH, Min DJ, Cho ML, Kim WU, Youn J, Park W, Cho CS, Kim HY. Shift toward T helper 1 cytokines by type II collagen-reactive T cells in patients with rheumatoid arthritis. Arthritis Rheum 2001; 44: 561-9.
57. Weiner HL, Zhang ZJ, Khouyr SJ, Miller A, Al-Sabbagh A, Brod SA, Lidor O, Higgins P, Sobel R, Nussenblatt RB, et al. Antigen-driven peripher-
al immune tolerance: suppression of organ-specific autoimmune diseases by oral administration of autoantigens. Ann N Y Acad Sci 1991; 636: 227-32.

58. Singh VK, Nagaraju K. Experimental autoimmune uveitis: molecular mimicry and oral tolerance. Immunol Res 1996; 15: 323-46.

59. Weiner HL, Friedman A, Miller A, Khoury SJ, Al-Sabbagh A, Santos L, Sayegh M, Nussenblatt RB, Trentham DE, Hafler DA. Oral tolerance: immunologic mechanisms and treatment of animal and human organ-specific autoimmune diseases by oral administration of autoantigens. Annu Rev Immunol 1994; 12: 809-37.

60. Lee MS. New insight on immune tolerance from transgenic mouse models. J Korean Med Sci 1996; 11: 1-7.

61. Corrigall VM, Bodman-Smith MD, Brunst M, Cornell H, Panayi GS. Inhibition of antigen-presentation cell function and stimulation of human peripheral blood mononuclear cells to express an antiinflammatory cytokine profile by the stress protein BiP: relevance to the treatment of inflammatory arthritis. Arthritis Rheum 2004; 50: 1164-71.

62. Luo X, Zuo X, Zhou Y, Zhang B, Shi Y, Liu M, Wang K, McMillian DR, Xiao X. Extracellular heat shock protein 70 inhibits tumour necrosis factor-alpha-induced proinflammatory mediator production in fibroblast-like synoviocytes. Arthritis Res Ther 2008; 10: R41.

63. Van Hervenijn MJ, Wieten L, van der Zee R, van Kooten PJ, Wagenaar-Hilbers JP, Hoek A, den Braber I, Anderton SM, Singh M, Meiring HD, et al. Regulatory T cells that recognize a ubiquitous stress-inducible self-antigen are long-lived suppressors of autoimmune arthritis. Proc Natl Acad Sci U S A 2012; 109: 14134-9.

64. Van Anken E, Romijn EP, Maggioni C, Mezghrani A, Sitaia R, Braakman I, Heck AJ. Sequential waves of functionally related proteins are expressed when B cells prepare for antibody secretion. Immunity 2003; 18: 243-53.

65. Lu MC, Lai NS, Yu HC, Huang HB, Hsieh SC, Yu CL. Anti-citrullinated protein antibodies bind surface-expressed citrullinated Grp78 on monocyte/macrophages and stimulate tumor necrosis factor alpha production. Arthritis Rheum 2010; 62: 1213-23.

66. Takano S, Ando T, Hiramatsu N, Kanayama A, Maekawa S, Ohnuma Y, Enomoto N, Ogawa H, Paton AW, Paton JC, et al. T cell receptor-mediated signaling induces GRP78 expression in T cells: the implications in maintaining T cell viability. Biochem Biophys Res Commun 2008; 371: 762-6.

67. Pino SC, O’Sullivan-Murphy B, Lidstone EA, Thornley TB, Jurczyk A, Lu MC, Lai NS, Yu HC, Huang HB, Hsieh SC, Yu CL. Anti-citrullinated protein antibodies bind surface-expressed citrullinated Grp78 on monocyte/macrophages and stimulate tumor necrosis factor alpha production. Arthritis Rheum 2010; 62: 1213-23.

68. Chang JS, Ocvirk S, Berger E, Kising S, Binder U, Skerra A, Lee AS, Haller P. Endoplasmic reticulum stress response promotes cytotoxic phenotype of CD8αβ+ intraepithelial lymphocytes in a mouse model for Crohn’s disease-like ileitis. J Immunol 2012; 189: 1520-10.

69. Zhang K, Shen X, Wu J, Sakaki K, Saunders T, Rutkowski DT, Back SH, Kaufman RJ. Endoplasmic reticulum stress activates cleavage of CREBH to induce a systemic inflammatory response. Cell 2006; 124: 587-99.

70. Xue X, Piao JH, Nakajima A, Sakon-Komazawa S, Kojima Y, Mori K, Yagita H, Okumura K, Harding H, Nakano H. Tumor necrosis factor alpha (TNFα) induces the unfolded protein response (UPR) in a reactive oxygen species (ROS)-dependent fashion, and the UPR counteracts ROS accumulation by TNFα. J Biol Chem 2005; 280: 33917-25.

71. Nakajima S, Hiramatsu N, Hayakawa K, Saito Y, Kato H, Huang T, Yao J, Paton AW, Paton JC, Kitamura M. Selective abrogation of BiP/GRP78 blunts activation of NF-κB through the ATF6 branch of the UPR: involvement of C/EBPα and mTOR-dependent dephosphorylation of Akt. Mol Cell Biol 2011; 31: 1710-8.

72. Ni M, Zhang Y, Lee AS. Beyond the endoplasmic reticulum: atypical GRP78 in cell viability, signalling and therapeutic targeting. Biochem J 2011; 434: 181-8.

73. Srikrishna G, Freeze HH. Endogenous damage-associated molecular pattern molecules at the crossroads of inflammation and cancer. Neoplasia 2009; 11: 615-28.

74. Davidson DJ, Haskell C, Majest S, Kherzai A, Egan DA, Walter KA, Schneider A, Gubbins EF, Solomon L, Chen Z, et al. Kringle 5 of human plasminogen induces apoptosis of endothelial and tumor cells through surface-expressed glucose-regulated protein 78. Cancer Res 2005; 65: 4663-72.

75. Katanasaka Y, Ishitii T, Asai T, Naitou H, Maeda N, Koizumi F, Miyagawa S, Ohashi O, Oku N. Cancer antineoovascular therapy with liposome drug delivery systems targeted to BiP/GRP78. Int J Cancer 2010; 127: 2685-96.

76. Dong D, Stapleton C, Luo B, Xiong S, Ye W, Zhang Y, Juveri N, Zhu G, Ye R, Liu Z, et al. A critical role for GRP78/BiP in the tumor microenvironment for neovascularization during tumor growth and metastasis. Cancer Res 2011; 71: 2848-57.

77. Paton AW, Beddow T, Thorpe CM, Whisthoch JC, Wilce MC, Rossjohn J, Talbot UM, Paton JC. AB5 subtilase cytotoxin inactivates the endoplasmic reticulum chaperoneBiP. Nature 2006; 443: 548-52.

78. Weidle UH, Maisel D, Klostermann S, Schiller C, Weiss EH. Intracellular proteins displayed on the surface of tumor cells as targets for therapeutic intervention with antibody-related agents. Cancer Genomics Proteomics 2011; 8: 49-63.

79. Lee SS, Joo YS, Kim WU, Min DJ, Min JK, Park SH, Cho CS, Kim HY. Vascular endothelial growth factor levels in the serum and synovial fluid of patients with rheumatoid arthritis. Clin Exp Rheumatol 2001; 19: 321-4.

80. Lu J, Kasama T, Kobayashi K, Yoda Y, Shirazawa F, Hanayuda M, Negishi M, Iide H, Adachi M. Vascular endothelial growth factor expression and regulation of murine collagen-induced arthritis. J Immunol 2000; 164: 5922-7.

81. Amano T, Yamasaki S, Yagishita N, Tsuchimochi K, Shin H, Kawahara K, Aratani S, Fujita H, Zhang L, Ikeda R, et al. Synoviolin/HRD1, an E3 ubiquitin ligase, as a novel pathogenic factor for arthropathy. Genes Dev 2003; 17: 2436-49.

82. Kikkert M, Doolman R, Dai M, Avner R, Hassink G, van Voorden S, Thaedar S, Roitelman J, Chau V, Wertz E. Human HRD1 is an E3 ubiquitin ligase involved in degradation of proteins from the endoplasmic reticulum. J Biol Chem 2004; 279: 3525-34.

83. Hu MC, Gong HY, Lin GH, Hu SY, Chen MH, Huang SJ, Liao CF, Wu JL, XBP-1, a key regulator of unfolded protein response, activates transcription of IGF1 and Akt phosphorylation in zebrafish embryonic cell line. Biochem Biophys Res Commun 2007; 359: 778-83.

84. Hampton RI. ER-associated degradation in protein quality control and cellular regulation. Curr Opin Cell Biol 2002; 14: 476-82.

85. Yamasaki S, Yagishita N, Tsuchimochi K, Nishioka K, Nakajima T. Rheumatoid arthritis as a hyper-endoplasmic-reticulum-associated degradation disease. Arthritis Res Ther 2005; 7: 181-6.

86. Yagishita N, Ohneda K, Amano T, Yamasaki S, Sugiuara A, Tsuchimochi K, Shin H, Kawahara K, Ohneda O, Ohta T, et al. Essential role of synoviolin in embryogenesis. J Biol Chem 2005; 280: 7909-16.