REVIEW
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Role of Endoplasmic Reticulum Stress in Rheumatoid Arthritis Pathogenesis

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Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by abnormal proliferation of synoviocytes, leukocyte infiltration, and angiogenesis. The endoplasmic reticulum (ER) is the site of biosynthesis for all secreted and membrane proteins. 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; Arhtritis; Rheumatoid

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_H17 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 (8-11). During ER stress, the glucose-regulated protein of 78 kDa (GRP78), a molecular chaperone also known as binding immunoglobulin protein (BiP), initiates a signaling cascade of UPR (9).

After initiation by GRP78, the main UPR signaling is propagated by three ER-localized protein sensors: inositol-requiring transmembrane kinase-endoribonuclease-1α (IRE1α), double-stranded RNA-dependent protein kinase-like ER kinase (PERK), and activating transcription factor 6 (ATF6) (12, 13). In the resting state, GRP78 binds the N-termini of IRE1α, PERK, and ATF6, preventing their activation. Upon activation, GRP78 binds to unfolded or misfolded proteins, and it releases IRE1α, PERK and ATF6, triggering UPR signaling. The intrinsic ribonuclease activity of IRE1α results in the production of X-box binding protein-1 (XBP-1), a transcription factor that induces the expression of genes involved in restoring protein folding or in degrading unfolded proteins (9, 14). On the other hand, PERK activates initiation factor 2α phosphorylation, preventing general protein synthesis through translation repression. However, under sustained ER stress, the cell may fail to resolve the protein-folding defect and to restore homeostasis in the ER; the resul-

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tant UPR then initiates programmed cell death, such as apoptosis and autophagy (12, 13).

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 spondyloarthropathies (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, phosphatidylinositide 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 synoviocyte 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* haploinsufficiency 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.

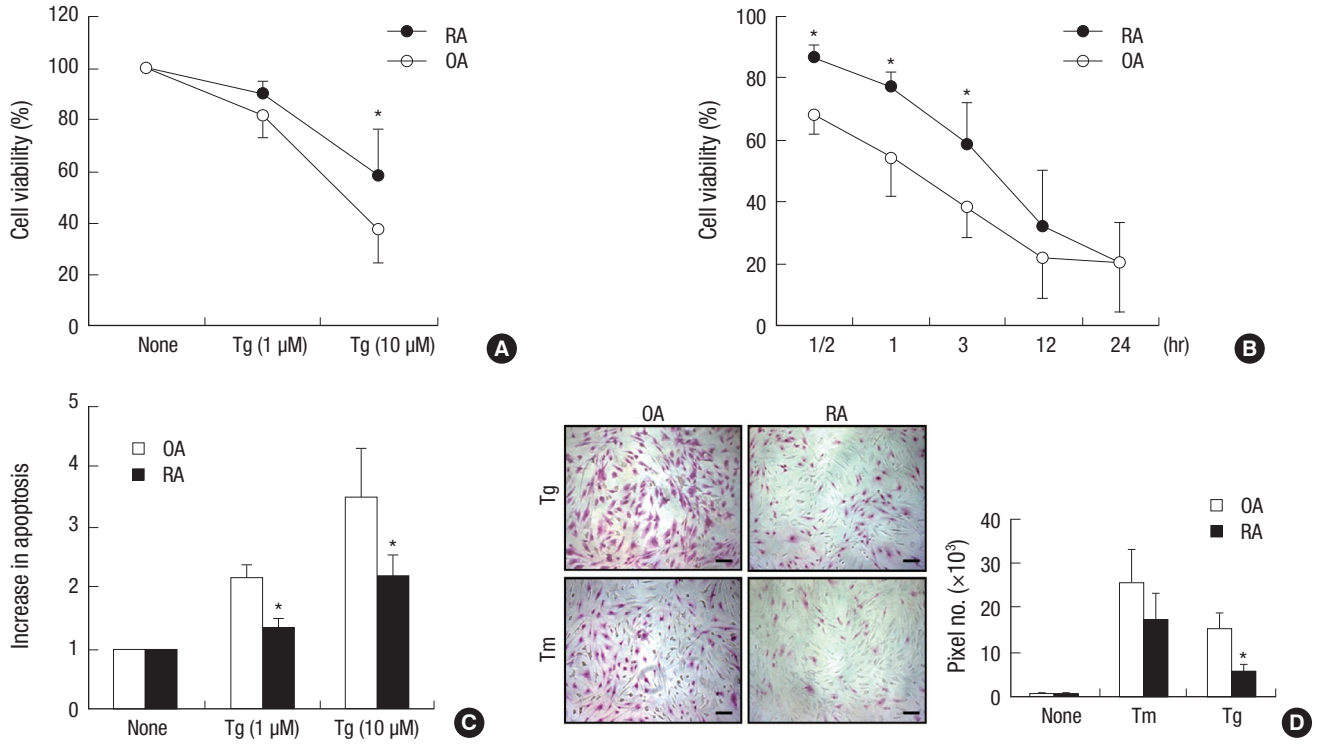

Fig. 1. Rheumatoid arthritis synoviocytes are less sensitive to ER stress-induced apoptosis than osteoarthritis synoviocytes. (**A** and **B**). FLS (4×10^3 cells) of RA (n = 5) and of OA patients (n = 5) were treated with tunicamycin (Tm) or thapsigargin (Tg). Cell viability was assessed by MTT (tetrazolium) assay. Data are the mean ± SD of five independent experiments performed in triplicate, and are presented as percentages versus untreated cells. *$P < 0.05$ compared with OA-FLS. (**A**) Cell viability determined 3 hr after treatment with thapsigargin (1 or 10 μM). (**B**) Time-dependent response to 10 μM of thapsigargin. (**C** and **D**). ER stress-induced synoviocyte apoptosis. (**C**) The apoptosis of the FLS (3×10^4 cells) of RA (n = 3) and of OA patients (n = 3) was induced by treating cells with thapsigargin (10 μM) or tunicamycin (20 μg/mL) for 1 hr. Degrees of apoptosis were assessed by cellular DNA fragmentation ELISA. Results are the means ± SD of three independent experiments, and are expressed as fold increases versus basal levels. *$P < 0.05$ compared with OA-FLS. (**D**) APOPercentage Apoptosis Assay. FLS were treated with thapsigargin (10 μM) or tunicamycin (20 μg/mL) for 2 hr. Apoptotic cells were bright pink (left panel). Representative digital images of three independent experiments are shown. Scale bars: 100 μm. Levels of apoptosis determined using APOPercentage apoptosis assay kits are also presented as pixel numbers using Adobe Photoshop (right panel). *$P < 0.05$ compared with OA-FLS. ER, endoplasmic reticulum; FLS, fibroblast-like synoviocytes; RA, rheumatoid arthritis; OA, osteoarthritis; SD, standard deviation; ELISA, enzyme-linked immunosorbent assay.

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

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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 T_H1 and T_H17 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 T_H1 and T_H17 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 T_H1 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 Ca^{2+} 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 CD8ab+ 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-in-

flammatory 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 cyto-chemokine 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

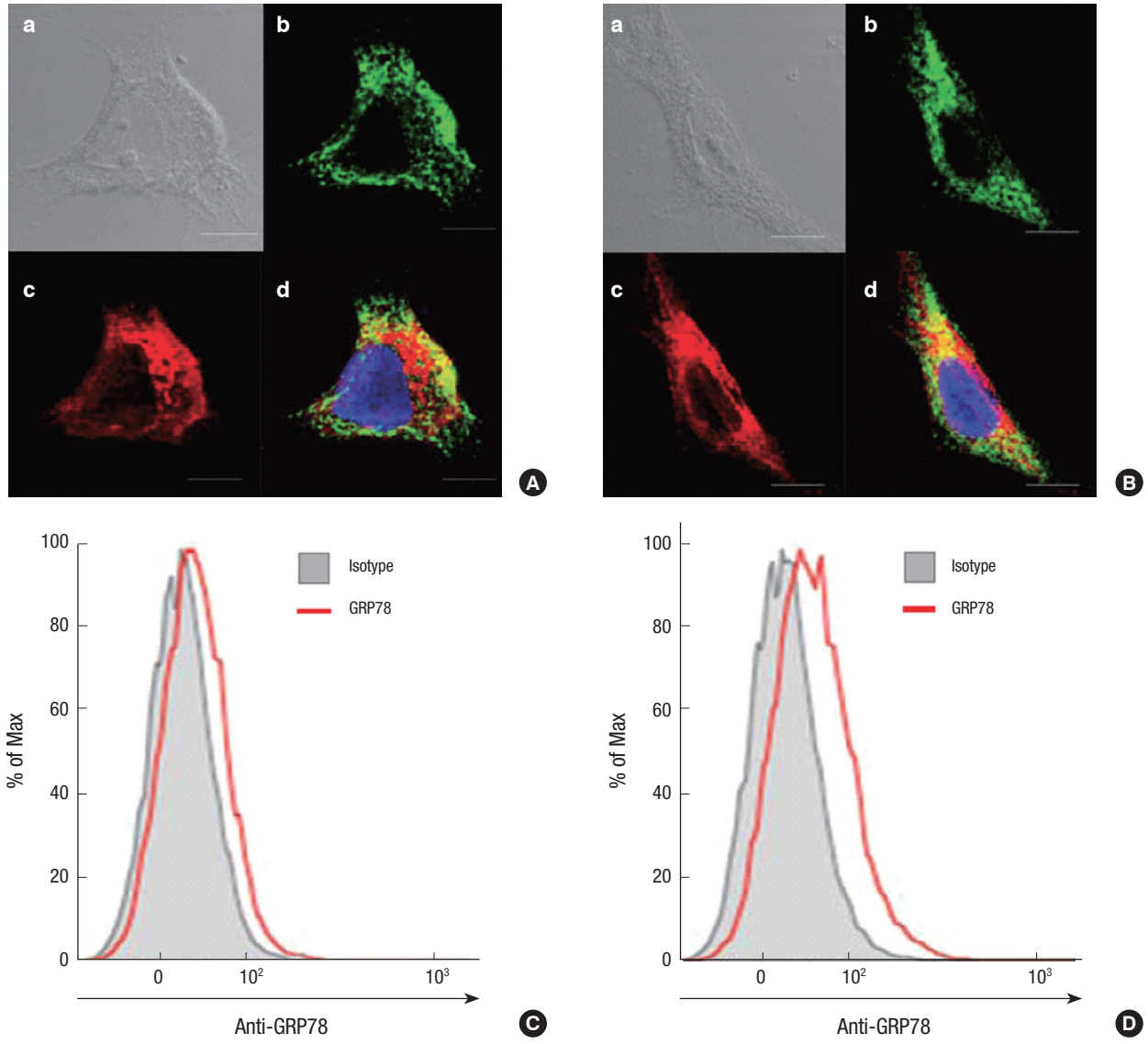

Fig. 2. GRP78 is expressed in the ER and membrane of synoviocytes. (**A** and **B**). Immuno-fluorescence staining of GRP78 in FLS. RA synoviocytes were permeabilized, and stained with anti-GRP78 antibody and CellLight ER-RFP, an ER marker. Images were obtained by confocal microscopy. (**a**) phase contrast 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.

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at times of acute or chronic physiological stress as a form of exosome, necrotic or apoptotic debris, can contribute to immune-modulating 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 Corrigall 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 lipopolysaccharides-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 ANGIOGENESIS

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

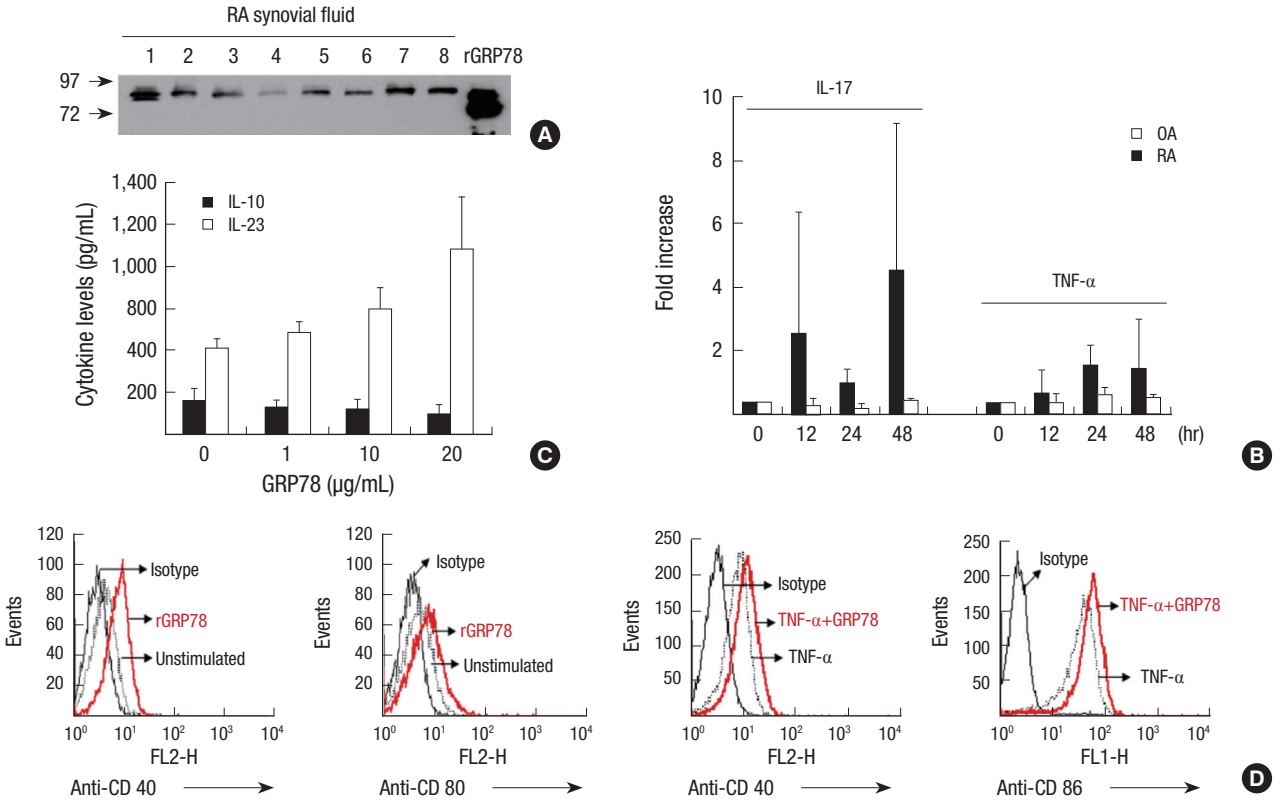

Fig. 3. Recombinant GRP78 induces pro-inflammatory response in rheumatoid mononuclear cells. (**A**) Expression of GRP78 in the synovial fluid of RA patients (n = 8), which was determined by Western blot analysis. (**B**) GRP78-induced production of IL-17 and TNF-α by synovial fluid mononuclear cells of RA patients (n = 3) versus peripheral blood mononuclear cells of OA (n = 3). Mononuclear cells (1×10^6) 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 (1×10^6) 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 (1×10^6) were stimulated with recombinant GRP78 (rGRP78) in the presence or absence of TNF-α (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; rGRP78, 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 therapeutic 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, $VEGF_{165}$-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 $VEGF_{165}$-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 co-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 ER 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

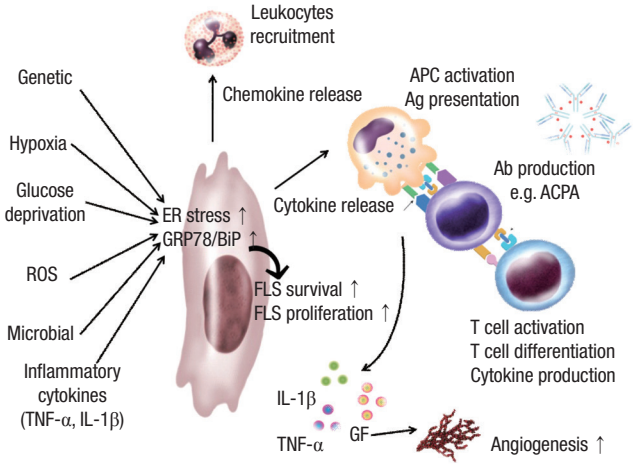

Fig. 4. Hypothetical model for the role of GRP78 in the pathogenesis of rheumatoid arthritis. GRP78, glucose-regulated protein of 78 kDa; ROS, reactive oxygen species; TNF-α, tumor necrosis factor-alpha; IL-1β, interleukin-1beta; ER, endoplasmic reticulum; BiP, binding immunoglobulin protein; FLS, fibroblast-like synoviocytes; APC, antigen presenting cell; Ag, antigen; Ab, antibody; ACPA, anti-cyclic citrullinated peptide antibodies; GF, growth factor.

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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 ACPA 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 ACPA, 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 synoviocyte 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 WIFPWIQL (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.

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