A pathogenic role for ER stress-induced autophagy and ER chaperone GRP78/BiP in T lymphocyte systemic lupus erythematosus

Won-Seok Lee,*1 Myung-Soon Sung,*1 Eun-Gyeong Lee,* Han-Gyul Yoo,† Yun-Hong Cheon,* Han-Jung Chae,† and Wan-Hee Yoo*,2

*Department of Internal Medicine, Chonbuk National University Medical School, and Research Institute of Clinical Medicine of Chonbuk National University Hospital, and Department of Pharmacology, Chonbuk National University Medical School, Jeonju, Jeonbuk, South Korea; and †Department of Pharmacy Practice, College of Pharmacy, University of Rhode Island, Kingston, Rhode Island, USA

ABSTRACT

Abnormal regulation of ER stress and apoptosis has been implicated in autoimmune disorders. Particularly, ER stress-induced autophagy and the role of GRP78, or BiP in T lymphocyte survival and death in SLE are poorly understood. This study investigated the pathogenic roles of ER stress-induced autophagy and GRP78/BiP in apoptosis of T lymphocytes. We compared spontaneous and induced autophagy and apoptosis of T lymphocytes in healthy donors and patients with SLE. The molecular mechanism of altered autophagy and apoptosis was investigated in T lymphocytes transfected with siRNA for beclin 1 and CHOP and T lymphocytes overexpressing GRP78. Decreased autophagy and increased apoptosis in response to TG-induced ER stress were observed in lupus T lymphocytes. GRP78 and ER stress-signaling molecules, such as PERK, p-eIF2α, IRE1, and ATF6 decreased, whereas CHOP levels increased in lupus T cells in response to TG. The levels antiapoptotic molecules, Bcl-2 and Bcl-XL, decreased, whereas the proapoptotic molecules, Bax and caspase 6, increased in lupus T cells. The TG-induced ER stress altered autophagy and apoptosis, which in turn, led to abnormal T cell homeostasis with increased apoptotic T cell death. We hypothesize that aberrant autophagy of T lymphocytes as a result of ER stress and decreased GRP78 expression is involved in the pathogenesis of SLE and might serve as important therapeutic targets.


Introduction

SLE is a systemic autoimmune disease of unknown etiology. The clinical manifestations visible in most organs are initiated by the production of autoantibodies to nuclear antigens and cell-surface and serum proteins [1, 2]. One of the most commonly implicated pathogenic mechanisms in SLE is impaired T lymphocyte homeostasis [3, 4].

Apoptosis regulates and maintains peripheral lymphocyte homeostasis. Therefore, immune disorders, such as immunodefiency and autoimmunity, emerge from the misregulation of lymphocyte apoptosis. Aberrant apoptosis, specifically increased T lymphocyte apoptosis, has been documented extensively in SLE [2, 5, 6]. Moreover, chronic lymphopenia [2] and compartmentalized autoantigen release [7] in SLE pathogenesis may be a result of the up-regulation of apoptosis of circulating T cells.

In addition to apoptosis, the role of autophagy in maintaining lymphocyte homeostasis has been reported previously [8–10]. Autophagy is a physiologic mechanism that promotes the turnover of cell macromolecules and organelles via lysosomal degradation, thereby regulating cell homeostasis, differentiation, and tissue remodeling. It exists in human and murine T lymphocytes [9, 11, 12] and has complex functions in T lymphocyte development, survival, and proliferation [8, 13]. Adverse environmental conditions, such as nutrient depletion and oxidative stress, can trigger autophagy by activating Atg proteins, such as microtubule-associated protein 1 LC3, a mammalian homolog of yeast Atg8 [13–16]. Endogenous LC3 is converted to its cytosolic form, LC3 I, which is then processed into lipidated LC3 II, which integrates into autophagosome membranes. Therefore, LC3 II levels are good indicators of the extent of autophagy.

Autophagy deregulation has been implicated in several human diseases, including autoimmune disorders [17, 18]. Genetic...
studies have identified mutations in autophagy regulators [11, 19], and both T cells from lupus-prone mice and patients with SLE show abnormal autophagy [18, 20]. The interaction of molecular pathways between autophagy and apoptosis is complex, and regulators of apoptosis activate autophagy [21–23]. However, how these interactions affect cell fate remains controversial, and there is a need to define the roles of ER stress-associated autophagy and apoptosis in T lymphocytes in patients with SLE.

A molecular chaperone, GRP78 or BiP, modulates ER function through protein folding and assembly, targeting misfolded protein for degradation, ER Ca2+-binding, and controlling the transduction of apoptotic signaling through protein folding and assembly, targeting misfolded protein for degradation, ER Ca2+-binding, and controlling the transduction of apoptotic signaling [24]. Because of its antiapoptotic property, stress induction of GRP78/BiP represents a principal prosurvival component of the UPR [25].

The aim of this study was to evaluate the pathogenic roles of ER stress-induced autophagy and ER chaperone GRP78/BiP in T lymphocyte survival and death in SLE. We found that ER-stress induced decreased autophagy, and GRP78/BiP expression led to increased CHOP-mediated apoptotic responses in T lymphocytes from patients with SLE compared with those donated by healthy donors. These results provide new insights into the pathogenic role of ER stress-induced autophagy and GRP78/BiP in the dysregulation of T lymphocyte homeostasis in SLE.

MATERIALS AND METHODS

Reagents and antibodies

TG was obtained from Sigma-Aldrich (St. Louis, MO, USA). mAb against LC3 II, Bcl-xL, Bax, Fas, PERK, ATF6, IRE1, p-eIF2α, GRP78, and CHOP were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA); beclin 1, caspase 6, and β-actin were purchased from Cell Signaling Technology (Danvers, MA, USA); and LC3 was obtained from ABR-Af-BioReagents (Thermo Fisher Scientific, Golden, CO, USA). CCK-8 was purchased from Dojindo Laboratories (Kumamoto, Japan). RPMI 1640 and FBS were obtained from Gibco-BRL (Life Technologies, Grand Island, NY, USA).

Isolation of PBMC and T lymphocyte sorting

A 30 to 40 ml peripheral blood sample was obtained from patients with SLE who fulfilled the American College of Rheumatology Criteria for SLE [26] and age- and sex-matched healthy donors, after provision of informed consent. The study protocol was approved by the Chungbuk National University Hospital Ethical Committee. Peripheral blood samples were diluted 1:2 with PBS (pH 7.4) and layered on Ficoll-Paque (Amersham Biosciences/GE Healthcare, Uppsala, Sweden) with centrifugation at 20°C for 30 min. PBMCs were collected from the interphase between the Ficoll-Paque and serum layers and washed twice with PBS.

T cells were isolated from PBMCs by a step of negative selection by use of a streptavidin-conjugated CD11b/macroage 1 antigen, CD16, CD19, CD36, CDH4a, CD56, and CD255a biotinylated antibody cocktail (Human Regulatory T Lymphocyte Separation Set; BD Biosciences, San Jose, CA, USA). In brief, PBMCs were incubated with cocktail antibodies at room temperature for 15 min and magnetic beads coupled with a mAb at room temperature for 30 min. T cells were purified by use of magnetic bead with >99% purity, as assessed by flow cytometric counting of T lymphocytes (data not shown).

Plasmid DNA and siRNA transfection of LC3-GFP, beclin 1, and CHOP

The cDNA encoding LC3-GFP was cloned into a retroviral vector (a gift from BinFeng Lu, University of Pittsburgh, Pittsburgh, PA, USA) with Thy-1.1 as a selection marker. To construct siRNA expression vectors, the following sequences were used: beclin 1, 5’-GATCCGCGAGTTGGCAGCAGATCTAAATATCATATGAGAGATTTGATTTGCTAAACTGTTTTTGGAAA-3’ and CHOP, 5’-AAGAAGCCAGCAAGGTCACAAG-3’, which were synthesized by Bioneer (Daejeon, South Korea). Primary resting human T cells were plated 24 h before transfection. Plated T cells were transfected with beclin 1 or CHOP and LC3-GFP fusion vector by use of the Amaxa transfection reagent (Lonza, Basel, Switzerland), according to the manufacturer’s protocol. Untransfected cells and nonsilencing, control, siRNA-transfected cells were used as negative controls. One day after transfection, the cells were recultured in 100 mm² dishes at 5 × 10^6 cells/dish. The transfection efficiency was >90%. Expression and silencing of the proteins listed above were assessed with Western blots.

GRP78 transfection

Transfection was performed according to Roche’s FuGENE HD protocol (Roche Applied Science, Indianapolis, IN, USA). In brief, primary resting T lymphocytes from healthy donors were cultured to 60–70% confluence in 6-well culture plates and transfected with 4 µg plasmid by use of 16 µl FuGENE HD (1:4 ratio). As a control, cells were transfected with vectors under the same conditions. Stable transfecants were selected in complete medium containing 400 µg/ml G418 and cultured for 2–5 weeks. G418-resistant clones were isolated and cultured in complete medium containing 200 µg/ml G418. Positive clones were identified by Western blot. The plasmids used in this experiment were pcDNA3.1 (+)-GRP78 and Rock dominant-negative recombiant pcAG-KDIA. pcDNA3.1 (+)-GRP78 recombinant was constructed by inserting a 2 kb fragment of human GRP78 cDNA into the HindIII/Xhol site of pcDNA3.1 (+) [27]. GRP78 overexpression was monitored by Western blot.

Cell viability analysis

Cell viability after TG treatment was determined by CCK-8 assay kits, according to the manufacturer’s instructions. In brief, 2-(2-methoxy-4-nitrophenyl)-5-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2Htetrazolium (CCK-8) was reduced by dehydrogenases in cells to yield an orange-colored product (formazan) [28]. The amount of formazan dye generated by intracellular dehydrogenases is directly proportional to the number of living cells. T cells were suspended at a final concentration of 1 × 10^6 cells/well and cultured in 96-well flat-bottomed microplates. After exposure to TG for 48 h, CCK-8 (10 µl) was added to each well of a 96-well, flat-bottomed microplate containing 100 µl culture medium and TG (0, 0.5, 1, 5, or 10 ng/ml), and the plates were incubated for 2 h at 37°C. Viable cells were counted by absorbance measurements at 450 nm by use of an automated microplate reader (Infinite M200; Tecan, Mannedorf, Switzerland). The OD at 450 nm was inversely proportional to the degree of cell apoptosis. Each independent experiment was performed in triplicate.

Annexin V and PI staining for apoptosis analysis

The appearance of phosphatidylserine on the extracellular side of the cell membrane was quantified by Annexin V/PI staining. After 5 ng/ml TG treatment for 24 or 48 h, control and TG-treated cells were stained with Annexin V-FITC (5 µl) and PI (10 µg/ml) for 10 min at room temperature, as recommended by the manufacturer. Cells were subjected to FACS analysis by use of a flow cytometer (BD Biosciences) with apoptotic cells as Annexin V positive/PI negative.

Immunoblotting

Sorted T cells (1 × 10^6 cells) were seeded on a 96-well, flat-bottomed microplate and harvested in PBS. After washing with PBS, cell pellets were lysed in lysis buffer (20 mM HEPES, pH 7.2, 1% Triton X-100, 150 mM NaCl, 0.1 mM PMSF, 1 mM EDTA, and 1 µg/ml aprotinin). After incubation for 30 min at 4°C, cellular debris was removed by centrifugation at 100,000 g for 30 min, and supernatants were analyzed by SDS-PAGE. Protein concentration was determined by the Bio-Rad protein assay reagent (Bio-Rad Laboratories, Hercules, CA, USA). Samples (50 µg) were prepared with 4 vol 0.5 M Tris buffer (pH 6.8) containing 4% SDS, 20% glycerol, and 0.05% bromophenol blue at 95°C for 5 min. SDS-PAGE was performed in 10% slab gels. Proteins were transferred to nitrocellulose membranes, which were then washed in blocking.
buffer (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 5% fat-free milk) for 60 min at room temperature with shaking and then washed with TBS with 0.01% Tween 20. Primary antibodies (10 μg/ml) were incubated at 4°C for 4 h, and secondary HRP-conjugated antibodies were incubated for 1 h. Reactive proteins were detected by use of ECL (Amersham Life Sciences/GE Healthcare).

**Quantification of autophagy**

T lymphocytes with LC3-GFP expression were fixed to slides with 2% paraformaldehyde and 0.1% Triton X-100 and counterstained with DAPI nuclear dye. Slides were examined by fluorescence microscopy. Cells were examined in more than 5 fields/slide on multiple slides. Cells with 2 or more visible autophagosomes were considered to be undergoing autophagy to allow for visualization error. The number of cells considered to be undergoing autophagy was divided by the total number of cells in the same field to determine the percentage of cells undergoing autophagy. Data represent the averages of all the fields.

**TEM**

T lymphocytes pellets were fixed in a 4% gluteraldehyde in 0.1 M sodium cacodylate buffer overnight. The samples were rinsed in 0.1 M cacodylate buffer containing 7.5% sucrose, 3 times for 15 min each and fixed in 1% osmium in cacodylate buffer for 1 h. After 3 washes in 0.11 M Veronal acetate buffer for 15 min each, the samples were incubated with 0.5% uranyl acetate in Veronal acetate buffer for 1 h at room temperature. Specimens were then dehydrated in increasing concentrations of ethanol (35, 70, and 95% and 2 changes of 100%) for 10 min each, followed by 2 changes of propylene oxide for 5 min each. The samples were incubated with a 1:1 mixture of 100% resin and propylene oxide for 1 h, followed by 2 changes of 100% resin (30 min each). Finally, the samples were embedded in resin and polymerized at 60°C overnight. Thick sections (0.5 μm) were cut and stained with toluidine blue for light microscopy selection of the appropriate area for ultrathin sectioning. Thin sections (60-90 nm) were cut, mounted on copper grids, and then stained with uranyl acetate and lead citrate. Micrographs were taken with a Philips LS 410 electron microscope (Philips, Amsterdam, The Netherlands). Autophagy was quantified by counting the cells with 2 or more visible autophagosomes, as described above.

**Statistical analysis**

Data are presented as the mean ± so of experiments performed in triplicate. All data were analyzed by the SPSS 12.0 program (SPSS, Chicago, IL, USA). Mean values were compared with Student’s t test or ANOVA as appropriate. Unpaired Student’s t tests were applied for assessing differences between healthy controls and SLE patients, whereas paired Student’s t tests were used for intergroup comparisons (basal vs. TG stimulation). Differences were considered significant at P < 0.05.

**RESULTS**

Changes of survival and apoptosis by TG in T lymphocytes

To evaluate whether there were any differences in survival and apoptosis after TG-induced ER stress, we examined the viability of peripheral T lymphocytes from patients with SLE and healthy donors by use of CCK-8 assays. T cell survival was decreased in a dose-dependent manner at 48 h after treatment with different concentrations (0, 0.5, 1, 5, 10 nM) of TG in both cells. This decrement of T cell survival following TG treatment was much higher in T lymphocytes of patients with SLE compared with healthy donors.

Next, we assessed the effects of TG-induced ER stress T lymphocyte apoptosis by use of flow cytometry with FITC-conjugated Annexin V and PI. As reported previously [5, 6], freshly isolated lupus T cells were more apoptotic in vitro than control T cells (Fig. 1B). In addition, we found that T lymphocytes from patients with SLE had significantly higher levels of apoptosis after TG stimulation compared with those from healthy donors (Fig. 1B). The percentage of apoptotic cells was not related to drug therapy and did not correlate with disease activity, as measured by the SLEDAI (data not shown).

Altered UPR-associated protein expression in T lymphocytes

When the client protein load exceeds the ER-folding capacity, it may result in the accumulation of misfolded proteins, causing ER stress. For a cell to overcome ER stress, there needs to be coordination between the client protein load and the capacity of the ER protein-folding machinery. This is achieved by the activation of several signaling pathways, collectively known as the UPR, which promotes an adaptive response to ER stress and re-establishes ER homeostasis [29]. UPR activation is mediated by 3 distinct ER stress sensors: IRE1, ATF6, and PERK, which activate downstream signaling effectors. To identify changes in the response to intracellular ER stress in SLE, we performed immunoblotting for these proteins in T lymphocytes from patients with SLE and from healthy donors following TG stimulation. As shown in Figure 2A, the expressions of PERK, IRE1, and ATF and the downstream signaling protein p-eIF2α were decreased in T lymphocytes from patients with SLE compared with those from healthy donors.

ER stress-induced apoptosis is regulated by anti- and pro-apoptotic signals, including Bcl-2, Bcl-XL, Bax, and caspases. To evaluate the roles of these signals in TG-induced ER stress-mediated apoptosis, we performed immunoblotting in peripheral T lymphocytes from patients with SLE and healthy donors after TG stimulation. We found that the levels of Bcl-2 and Bcl-XL were decreased, and the expressions of Bax and caspase 6 were increased in T cells from patients with SLE compared with healthy donor T cells (Fig. 2B).

**Autophagy induction by TG in T lymphocytes**

We took 2 approaches to determine whether autophagy was affected by TG stimulation in isolated peripheral T lymphocytes from patients with SLE and healthy donors. First, to determine if T lymphocytes express autophagy machinery, peripheral T cells were sorted (>99% pure), and immunoblotting was performed to detect the expression of LC3 II (yeast Atg8) and beclin 1. Second, we infected T cells with a retroviral construct that allowed us to express an LC3-GFP fusion protein in the target cells that could be examined with fluorescence microscopy. The time required for peripheral lymphocyte isolation was constant (2–4 h) in all subjects, and biologic samples were isolated and studied immediately after blood drawing.

The baseline expression levels of beclin 1 and LC3 II in T cells from patients with SLE were compared with T cells from age- and sex-matched healthy controls (data not shown). After TG stimulation, the level of beclin 1 and the LC3 II/LC3 I ratio were decreased in T cells from patients with SLE compared with those from healthy donors (Fig. 3A). We also found that LC3-GFP fusion protein expression and the percentage of autophagic cells were decreased in T cells from patients with SLE compared with those from healthy donors at baseline and after TG stimulation.
We then used TEM to determine if double-membrane autophagosomes were present in T lymphocytes from patients with SLE and healthy donors (Fig. 3C). Quantification revealed that there were significantly fewer autophagosome-positive cells in patients with SLE compared with those from healthy donors (Fig. 3C). Because some patients with SLE were treated with the antimalarial drug hydroxychloroquine, which is a potent inhibitor of autophagy [30], we evaluated our results with this in mind. However, no difference was found between patients treated with or without hydroxychloroquine. In addition, no significant correlations between autophagy and SLE clinical features were observed (data not shown).

Relationship of beclin-1 with apoptotic responses and apoptosis-related protein expression in TG-stimulated T lymphocytes

Although autophagy is important for survival during periods of nutrient deprivation, its role in regulating cellular life and death...
attenuated autophagic reaction is associated with T lymphocyte cell death in T lymphocytes after TG stimulation, we used siRNA to knock down the expression of beclin 1, which is crucial for autophagy [33]. We generated T cells with vectors expressing beclin 1 siRNA. Beclin 1 expression and the ratio of LC3 II:LC3 I were decreased drastically before (data not shown) and after TG stimulation in T cells expressing beclin 1 siRNA constructs compared with T cells from healthy donors and those expressing control beclin 1 siRNA (Fig. 4B). In addition, we cultured these cells and examined whether reduced autophagic reactions were associated with TG-induced, ER stress-mediated apoptosis. Consistent with the hypothesis that autophagy affects TG-induced, ER stress-mediated apoptosis of T cells, T lymphocytes expressing beclin 1 siRNA constructs were much more susceptible to TG-induced apoptosis than healthy donor T cells and those with control beclin 1 siRNA (Fig. 4C). Therefore, autophagy mediates TG-induced, ER stress-mediated T cell apoptosis by affecting the expression of apoptosis-related proteins.

**TG-induced, ER stress-mediated CHOP and GRP78 expression in T lymphocytes**

Depending on the duration and amplitude of ER stress, cells initiate survival or apoptotic pathways to return the cell to homeostasis [34, 35]. We assessed these pathways in peripheral T lymphocytes from patients with SLE and healthy donors by immunoblotting for GRP78, a central regulator of UPR, and CHOP protein, which represses the promoter of the bcl-2 gene, after stimulation with TG. GRP78 and CHOP expression was decreased and increased, respectively, in peripheral T lymphocytes from patients with SLE compared with those from healthy donors (Fig. 5A).

**Relationships of CHOP and GRP78 with apoptosis-related proteins and apoptotic responses**

To determine the role of the ER chaperone CHOP in apoptosis induced by TG-induced, ER stress in T lymphocytes, we performed immunoblotting to assess the apoptosis-related proteins (Bcl-2, Bcl-X$_L$, and Bax) in control, CHOP siRNA knockdown, and GRP78-overexpressing T cells. The levels of Bcl-2 and Bcl-X$_L$ were increased, and the expression of Bax was decreased in T cells expressing CHOP siRNA constructs compared with both healthy donor T cells and those with control CHOP siRNA (Fig. 5B). In addition, Bcl-2 and Bcl-X$_L$ expression was decreased, and Bax expression was increased in T cells overexpressing GRP78 compared with healthy donor T cells and those transfected with control vector (Fig. 5C). To evaluate the roles of CHOP and GRP78 in apoptotic cell death of T lymphocytes after TG stimulation, we performed siRNA knockdown of CHOP and GRP78 overexpression with transfection. T lymphocytes with CHOP siRNA knockdown (Fig. 6A) or GRP78 overexpression (Fig. 6B) were less susceptible to apoptosis before and after treatment with TG than healthy donor T cells and those with control CHOP 1 siRNA or those transfected with control vector (Fig. 6A and B). These results suggest that dysregulation of TG-induced, ER stress-mediated apoptosis of peripheral T lymphocytes by the ER chaperones GRP78 and CHOP via anti- and proapoptotic signals, including Bcl-2, Bcl-X$_L$, and Bax, might be involved in SLE pathogenesis.

**DISCUSSION**

Our data support the hypothesis that ER stress and ER stress-induced autophagy are crucial to the apoptosis and survival of T lymphocytes in SLE. Therefore, the modulation of ER stress can be a valuable pharmacologic target for novel therapies for SLE.
The peripheral pool of T lymphocytes reflects a tight balance of thymic efflux, survival/death, and proliferation in the adaptive immune system, which stabilizes and limits the T cell population. The SLE T cells are integral in their pathogenesis, and deregulation of T lymphocyte homeostasis is one of the most implicated pathogenic mechanisms in SLE [3, 4]. Environmental factors (e.g., ultraviolet light, viral infections) that cause lupus flare-ups induce ER stress-induced cell death and anti-dsDNA antibody production [36, 37]. In this study, we analyzed whether there were alterations in T cell homeostasis in SLE by evaluating the survival and apoptotic responses to TG-induced ER stress. Following TG stimulation, the T lymphocytes from patients with SLE had significantly higher levels of apoptosis and reduced survival compared with that of healthy donors. These results were not influenced by drug therapies, as the disease activity was measured by the SLEDAI (data not shown). To evaluate the roles of ER stress sensors, such as IRE1, ATF6, and PERK, in SLE, we quantified their expression levels and found decreased reduction in T cells from patients with SLE. Likewise, a recent study found that the down-regulation of ER stress sensors in SLE patients was linked to the abnormal UPR, which may contribute to SLE pathogenesis [38].

Although the role of autophagy in cell death is controversial, it may regulate several T cell functions and affect T lymphocyte homeostasis by modulating apoptosis, survival, and proliferation [8, 22]. Thus, we chose to focus on the differences in autophagy in T cells from patients with SLE. We evaluated protein markers for autophagy (beclin 1 and LC3 II) by immunoblotting and quantifying autophagosome-positive T cells by TEM. TG-induced, ER stress-mediated autophagy decreased in T cells from patients with SLE compared with that from healthy controls. These results suggested that the reduced responses of ER stress sensors associate with a decreased autophagic response to ER stress.

Although autophagy and apoptosis are markedly different processes, several pathways regulate autophagic and apoptotic machinery. Autophagy can cooperate with apoptosis, and the regulation mechanisms of autophagy and apoptosis are probably linked at the molecular level [39]. Members of the beclin 1 and Bcl-2 family could serve as a point of communication between the autophagic and apoptotic pathways. Beclin 1 was identified originally as a Bcl-2-interacting protein [34]. It interacts directly with Bcl-2 and other antiapoptotic Bcl-2 family proteins, such as Bcl-XL [40]. Bcl-2 inhibits beclin 1-dependent autophagy in yeast and mammalian cells.

To investigate the possible linkage between ER stress-induced autophagy and T cell apoptosis, the expression levels of beclin 1, LC3 II, Bcl-2, Bcl-XL, Bax, and caspase 6 were assessed in T cells expressing beclin 1 siRNA after TG-induced ER stress induced by

Figure 3. ER-stress-induced autphagic responses in T cells. (A) To evaluate autophagic reactions to ER stress, the expression of the autophagy genes beclin 1 and LC3 II were analyzed with immunoblotting after stimulation with TG (5 ng/ml) for 48 h. β-Actin serves as a loading control. (B) The cDNA encoding LC3-GFP was cloned into a retroviral vector with Thy-1.1 as a selection marker. T lymphocytes were transduced with retrovirus containing LC3-GFP. These cells were harvested and examined for autophagosomes by fluorescence microscopy. (Left) A cell expressing LC3-GFP without autophagosomes. (Right) A cell undergoing autophagy after TG stimulation. The numbers of autophagosomes were quantified by fluorescence microscopy. Data are the percentages of autophagic T lymphocytes and are expressed as the mean ± so of 3 independent experiments. *P < 0.05, paired Student’s t-test versus no TG; †P < 0.05, unpaired Student’s t-test compared with healthy controls. (C) To evaluate the autophagic phenomenon in response to ER stress, TEM micrograph analyses were performed by use of sectioned lymphocytes. Autophagy was analyzed after 12 h stimulation with TG (5 ng/ml) by counting the autophagosome-positive T cells (original scale bars, 0.5 μm). The values are the mean ± so percentages of autophagosome-positive cells of 3 independent experiments. *P < 0.05, paired Student’s t-test compared with condition without TG; †P < 0.05, unpaired Student’s t-test compared with healthy controls.
The expression levels of Bax and caspase 6 increased in TG-stimulated T cells with beclin 1 siRNA by stimulation with TG, which supports the role of Bax and caspase 6 in triggering and mediating ER stress-induced apoptosis [31]. Bcl-2 and Bcl-XL decreased in response to TG in T cells expressing beclin-1 siRNA, whereas the number of apoptotic T cells increased significantly. These results suggest a link between autophagy and apoptosis during ER stress conditions in T cells. We observed lower levels of autophagy and concomitant, higher levels of apoptosis, suggesting that cooperation between autophagic and apoptotic machinery might be an important pathogenic mechanism in SLE.
The 78 kDa GRP78 is a protein-folding chaperone that acts as the central regulator of the ER stress response. Induced by various stress factors, it is crucial in maintaining T cell viability through controlled apoptosis and cell proliferation [40]. In a recent study, GRP78 deficiency attenuated granzyme B-mediated cytotoxicity and reduced T cell proliferation in T cells, suggesting that GRP78 regulates T cell function [41]. The regulation of T cell survival under ER stress was previously associated with the induction of CHOP protein expression, which is involved in ER stress-induced apoptosis [39, 42]. We examined GRP78 and CHOP protein levels in T cells from patients with SLE after stimulation with TG to assess their roles in T cell survival. GRP78 and CHOP expression levels decreased and increased, respectively, in peripheral T lymphocytes from patients with SLE compared with those from healthy donors. Furthermore, apoptosis-associated proteins displayed higher Bcl-2 and Bcl-Xs expression and lower expression of Bax in response to TG in T cell CHOP knockdown or GRP78 overexpression. Our data suggest that GRP78 responses in SLE patients may confer higher levels of T cell apoptotic responses during ER stress, leading to a decrease in T lymphocytes. However, the exact role of GRP78 in T cell activation, differentiation, proliferation, and survival in T cells is unclear. Thus, the determination of the necessity of intracellular GRP78 in T cell pathophysiology and the development of T cell-dependent autoimmune disease, such as SLE, may aid in the development of a diagnostic marker or therapeutic target.

There were several limitations of this study. We were unable to assess the precise roles of autophagic and apoptotic T cells in SLE immunopathogenesis. T cells are critical to the immune regulation in SLE, as they activate B cells and stimulate autoantibody production. However, we only focused on T cell survival; the functionality of these cells was not examined. In addition, we did not evaluate the effects of ER stress on autophagic and apoptotic responses in different subsets of T cells. A study reported that CD4+ T cells have elevated levels of apoptosis in active SLE patients compared with normal controls, and PBMCs cultured with SLE patient sera exhibited increased apoptosis of CD3', CD4', and CD8' T cells but not CD19' B cells [5]. Different T cell subsets from SLE patients can give different results, thus verification of possible differences between effectors and memory T cells on autophagy and apoptosis following ER stress is required. Another limitation is the absence of control groups with other rheumatic diseases. Increased lymphocyte apoptosis has been described in patients with vasculitis, undifferentiated connective tissue diseases, and rheumatoid arthritis [43]. Although there were no significant differences in the autophagic and apoptotic responses of T cells in SLE patients with different clinical characteristics, we cannot entirely exclude the possible influence of clinical parameters, such as disease activity and therapeutic immunosuppressive drugs. Finally, this study is severely limited by the nonphysiologic stimulation of ER stress in the cells. The ER stress response regulates cell survival by inducing autophagic or apoptotic responses in proportion to the intensity of the stress [44].

In summary, aberrant UPRs were found in patients with SLE, displaying down-regulation of ER stress sensors and decreased levels of autophagy. ER stress also correlated with higher levels of CHOP-mediated apoptotic responses in T lymphocytes of the SLE patients. Our results suggest that lower GRP78 responses in T cells during ER stress led to higher levels of apoptosis in SLE patients and lower levels of T lymphocytes. A better understanding of how proteins are modulated in response ER stress and its pathophysiological processes may elucidate the mechanism of SLE development and provide insight into the development of novel therapeutics.

**AUTHORSHIP**

All authors were involved in drafting or critical revising of the intellectual content in the article, and all authors approved the final version. W.-H.Y. had full access to the data in the study, takes responsibility for the integrity of the data and the accuracy of the data analysis, and provided the study conception and design. Y.-H.C., M.-S.S., and E.-G.L. completed data acquisition. W.-S.L., H.-G.Y., H.-J.C., and W.-H.Y. did the data analysis and interpretation.
ACKNOWLEDGMENTS

Chonbuk National University Hospital Research Institute of Clinical Medicine supported this study. The authors thank all of the members of the Institute of Bone and Joint Diseases at the Chonbuk National University.

DISCLOSURES

The authors declare no conflict of interest.

REFERENCES

27. Lee et al. ER stress and T lymphocyte homeostasis in SLE

KEY WORDS:

apoptosis, SLE, T cells
A pathogenic role for ER stress-induced autophagy and ER chaperone GRP78/BiP in T lymphocyte systemic lupus erythematosus


J Leukoc Biol 2015 97: 425-433 originally published online December 16, 2014
Access the most recent version at doi:10.1189/jlb.6A0214-097R

References
This article cites 44 articles, 11 of which can be accessed free at: http://www.jleukbio.org/content/97/2/425.full.html#ref-list-1

Subscriptions
Information about subscribing to Journal of Leukocyte Biology is online at http://www.jleukbio.org/site/misc/Librarians_Resource.xhtml

Permissions
Submit copyright permission requests at: http://www.jleukbio.org/site/misc/Librarians_Resource.xhtml

Email Alerts
Receive free email alerts when new an article cites this article - sign up at http://www.jleukbio.org/cgi/alerts