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The Endoplasmic Reticulum Stress-Inducible Protein, Herp, Is a Potential Triggering Antigen for Anti-DNA Response

Yasuhiko Hirabayashi,*† Yumiko Oka,* Tomoko Ikeda,* Hiroshi Fujii,* Tomonori Ishii,* Takeshi Sasaki,*† and Hideo Harigae*

Anti-dsDNA Abs are highly specific indicators of systemic lupus erythematosus (SLE) and play a pathogenic role in lupus nephritis. Human anti-dsDNA Abs are most likely generated by an Ag-driven mechanism. However, the Ag responsible for triggering anti-dsDNA Ab production has not been identified. To search for proteins that are cross-reactive with anti-dsDNA Abs, we screened a cDNA library from a patient with SLE and showed that a mixture of Ags can be immunogenic in vivo. Thus, the trigger of anti-DNA response is closely related to the pathogenesis of SLE.

We have shown previously that the clonotypes of anti-DNA Abs are marked heterogeneous in the circulation (2), but restricted in those eluted from the renal glomeruli of patients with lupus nephritis. Moreover, high-affinity binding to dsDNA is acquired by somatic mutations (3). These observations indicate that human nephritogenic anti-DNA Abs may be restricted despite the presence of many clonotypic Abs in the circulation (4) and are most likely generated by an Ag-driven mechanism. However, stimulation with mammalian native DNA failed to evoke the synthesis of antibody to DNA in vivo or in vitro, suggesting that DNA itself does not act as a triggering or driving Ag (5). The origin of anti-DNA Abs is a long-standing enigma.

Anti-DNA responses can be evoked by DNA with the aid of a carrier, such as the 27-amino acid nucleic acid-binding Fus1 peptide (6) or polyoma BK virus large T Ag (7). DNase I—dsDNA complex (8), nucleosomes, and crude histones (9) have also been shown to induce production of IgG isotype anti-DNA Abs in mice, suggesting a possible role of excess amounts of DNA—protein complex in disruption of tolerance to DNA.

Another possible mechanism is molecular mimicry. Some mouse or human anti-DNA mAbs have been shown to cross-react with nonnucleic acid self-Ags, such as extracellular matrix protein HP8 (10), heterogeneous nuclear ribonucleoprotein A2 (11), NR2 glutamate receptor (12), α-actinin (13, 14), and phospholipids, including cardiolipin (15). However, it is not yet known whether these molecules can elicit anti-DNA responses in vivo or in vitro. It should be noted that immunization with the peptide DWEYSVWLSN, which is recognized by the R4A mouse anti-dsDNA mAb, elicited anti-dsDNA Ab production and caused deposition of IgG in glomeruli in normal mice (16). These observations indicate that a nonnucleic acid Ag can elicit production of anti-DNA Abs and cause renal disorder in normal animals. However, no proteins containing this peptide sequence have been reported to date.

We prepared the O-81 human anti-DNA mAb, which binds strongly to ssDNA and moderately to dsDNA, and demonstrated that O-81 Id is distributed among IgG anti-DNA Abs of circulating immune complexes as well as lupus glomeruli deposits (17–20). The i.v. infusion of IgG isotype anti-DNA Abs expressing O-81 Id also caused glomerular IgG deposition in SCID mice (21). The VH region of O-81 Ab contains many somatic mutations (22). Similarly, the VH regions of O-81 Id-positive B cells in patients with SLE were shown to already contain somatic mutations (23).

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Materials and Methods

Patients

All subjects gave their informed consent to participation in the study, which was approved by the Ethics Committee of Tohoku University. The classification criteria of the American College of Rheumatology were used for diagnosis of SLE (24) (Supplemental Table I).

Two-hybrid assay

VH and Vκ genes of the O-81 clone were cloned as described previously (22). The Vκ gene of O-81 was amplified by PCR with two primers (5'-GGAAGATCTGGTGGAGGTGGATCAGAGGTGCAGCTGGTGGAG-3' and 3'-GAAAAGCTTGCGGCCGCTGATGTTGTGATGACTCAGGAGGTCGCTCTCGG-5') digested with HindIII and BamHI, and inserted into the corresponding sites of the pTRG target vector (pTRG-SfiI). A cDNA library was made from the corresponding sites of the pTRG target vector (pTRG-SfiI). Approximately 1.4 × 106 transformants were isolated from this cDNA library and ligated into the corresponding sites of the pTRG target vector (pTRGSfiI). A DNA library was made from the PBLs obtained from a patient with active lupus nephritis using a MicroFast Track mRNA isolation kit (Invitrogen, Carlsbad, CA) and a SMART cDNA library construction kit (BD Clontech, Palo Alto, CA). The cDNA library was digested with SfiI and ligated into the corresponding site of the pTRG-SfiI. Approximately 1.4 × 10<sup>9</sup> Escherichia coli transformants were screened for cDNA clones encoding polypeptides that interacted with the O-81 single-chain Fv using the BacterioMatch Two-Hybrid System (Stratagene, La Jolla, CA). To reduce the background, the concentration of carbencillin was increased from 250 μg/ml in steps to 500 μg/ml.

Ag and Ab

Recombinant sHerp protein, an alternative splice variant of homocysteine-induced endoplastic reticulum protein (Herp) followed containing the first six exons of Herp (25, 28). Recombinant GST protein was produced in the same way using pGEX6P1 plasmid (GE Healthcare, Buckinghamshire, U.K.). Oligonucleosomes were prepared from chicken erythrocytes as described previously (26). Soluble chromatin was obtained by digestion of nuclei with micrococcal nuclease (Sigma-Aldrich, St. Louis, MO) and fractionated into oligomers by gel filtration on Sephacryl S-400 (GE Healthcare). To measure the membrane fraction of EBV-transformed cells, the cells were suspended in ice-cold buffer containing 10 mM HEPES (pH 7.4), 250 mM sucrose, 10 mM KCl, 1.5 mM MgCl<sub>2</sub>, 1 mM EDTA, and 1 mM EGTA. The lysates were centrifuged at 1000 × g for 5 min at 4°C, and the supernatants were centrifuged at 100,000 × g for 5 min at 4°C. The pellets were resuspended in PBS (pH 7.4).

Sera from SLE patients were dialyzed against PBS containing 10 mM MgCl<sub>2</sub> and incubated with 100 μg/ml DNAse I for 1 h at 37°C to increase the recovery rate of anti-DNA Abs from the sera (27). The IgGs from DNAse I-treated sera purified with protein A/G (Pierce, Rockford, IL) were further purified as IgG anti-dsDNA Abs using a native DNA-cellulose column (GE Healthcare) or as IgG anti-Herp Abs using an sheep column, which was made by immobilization of sHerp on HitTrap NHS-activated HP (GE Healthcare).

ELISA

For dsDNA ELISA, calf thymus DNA (Invitrogen) was treated with S1 nuclease (Takara Bio, Otsu, Japan) to remove ssDNA and then digested with or Abs in PBS-T for 1 h at room temperature. After washing, the plates were incubated for 1 h at 37°C with HRP-conjugated secondary Abs and washed again. To develop the assays, 3’,5’-tetramethyl benzidine substrate solution (Pierce) was added at room temperature. The reaction was terminated by adding NH<sub>2</sub>S<sub>2</sub>O<sub>4</sub> and absorption at 450 nm was determined. All assays were performed at least in triplicate. Data represent means of experiments performed in triplicate, and error bars represent the SEM. Statistical significance was determined by the two-tailed Student t test, with p < 0.05 indicating significance.

Immunoﬂuorescence assay

Cells were fixed in 50% acetone and 50% methanol for 20 min at −20°C and blocked with 5% normal goat serum and 3% BSA in PBS overnight at 4°C. To detect Herp, the PBLs were incubated with the HT2 mouse IgG<sub>1</sub> anti-human Herp mAb (25) or mouse IgG<sub>1</sub> (Pierce) for 1 h. For nuclear Ab assay, Hep-2 cells were fixed, blocked, and incubated with diluted mouse sera (1:300) for 2 h. Bound mouse IgGs were detected using FITC-conjugated goat anti-mouse IgG Ab (Kirkegaard & Perry Laboratories, Gaithersburg, MD; Fig. 2).

HeLa cells were cultured with 1 μM thapsigargin for 6 h. After washing, the cells were cultured overnight. The cells were blocked and stained with Qdot655-conjugated HT2 Ab or with Qdot655-conjugated mouse IgG<sub>1</sub> for 1 h at room temperature without fixation. (Fig. 5A).

EBV-transformed B cells were fixed in 50% acetone and 50% methanol for 20 min at −20°C and blocked with 5% normal goat serum and 3% BSA in PBS overnight at 4°C. The cells were co-stained with the HT4 mouse IgG<sub>2a</sub> anti-human Herp mAb, which we made previously, and mouse IgG<sub>1</sub> anti-BZLF1 mAb (DakoCytomation, Carpinteria, CA) for 1 h at room temperature followed by co-staining with rhodamine-conjugated goat anti-mouse IgG<sub>2a</sub> Ab (Santa Cruz Biotechnology, Santa Cruz, CA) and FITC-conjugated goat anti-mouse IgG<sub>1</sub> Ab for 1 h at room temperature (Santa Cruz Biotechnology; Fig. 5B).

To detect apoptosis, caspase activity was visualized as a green color using an FAM ELICA poly-capase assay kit (Immunochemistry Technologies, Bloomington, MN) or phosphatidylinerse translocated to the cell surface was stained with an ApoAlert annexin V-FITC apoptosis kit (Clonotech). Cell nuclei were stained with Hoechst, followed by staining with Qdot655-conjugated HT2 Ab or with Qdot655-conjugated mouse IgG<sub>1</sub> for 1 h at room temperature. Qdot655-conjugated Abs were made using a Qdot655 Ab conjugation kit (Invitrogen; Fig. 3, Supplemental Fig. 1).

Immunofluorescence histochemistry

Fresh-frozen tissue sections 4 μm thick were fixed in 100% acetone for 10 min at 4°C and blocked with 5% normal goat serum and 3% BSA in PBS overnight at 4°C. Sections were stained with FITC-conjugated goat F(ab’)<sub>2</sub> anti-mouse IgG Ab (Kirkegaard & Perry Laboratories) for 1 h at room temperature.

Mice and immunization

Groups of ten 6-wk-old female BALB/c mice were immunized i.p. with 100 μg sHerp or 100 μg hen egg lysozyme (HEL, Sigma-Aldrich) in Ribi adjuvant R-700 (Corixa, Seattle, WA) on days 0 and 14, followed by immunization with 50 μg HEL or 50 μg HEL every 2 wk until day 126. Blood was collected from the tail vein of each mouse. Mice were sacrificed on day 136 after initial immunization (Fig. 4A–4C).

Groups of five 6-wk-old female BALB/c mice were immunized i.p. with 100 μg sHerp, 100 μg GST, or 10 μg oligonucleosomes without adjuvant on days 0 and 10, followed by immunization with 50 μg sHerp, 50 μg GST, or 10 μg oligonucleosomes on day 20. Blood was collected on day 23 (Fig. 4D).

Groups of eight 6-wk-old female BALB/c mice were immunized i.p. with the membrane fraction prepared from 5 × 10<sup>6</sup> EBV-transformed cells for each mouse for each time point without adjuvant on days 0, 14, 28, 42, and 56. Mice were sacrificed on day 63 after initial immunization (Fig. 5C–5E).

Results

Cross-reactivity of lupus anti-DNA Abs to Herp

To identify the proteins that may be recognized by the O-81 human anti-DNA mAb, the O-81 single-chain Fv was used as bait in a two-hybrid screen of a cDNA library from a patient with active lupus nephritis. After several rounds of screening, a transcript was isolated that was shown to be derived from the Herp gene (28). This transcript contained the first six exons of Herp followed by part of 0.05% Tween-20 (PBS-T) and then incubated with serum or Abs in PBS-T for 1 h at room temperature. After washing, the plates were incubated for 1 h at 37°C with HRP-conjugated secondary Abs and washed again. To develop the assays, 3’,5’-tetramethyl benzidine substrate solution (Pierce) was added at room temperature. The reaction was terminated by adding NH<sub>2</sub>S<sub>2</sub>O<sub>4</sub> and absorption at 450 nm was determined. All assays were performed at least in triplicate. Data represent means of experiments performed in triplicate, and error bars represent the SEM. Statistical significance was determined by the two-tailed Student t test, with p < 0.05 indicating significance.
the intronic sequence 3’ of exon 6, indicating that it is an alternative splice variant of Herp (designated sHerp). The putative sHerp protein of 302 aa terminates in a predicted transmembrane domain.

The original O-81 mAb shows strong binding to ssDNA (29); however, it also shows moderate binding to both dsDNA and recombiant sHerp (Fig. 1A). In a competitive inhibition ELISA, binding of O-81 Ab to sHerp was blocked almost completely by ssDNA but not by dsDNA (Fig. 1B). Alternatively, the binding of O-81 Ab to dsDNA was moderately blocked by sHerp, whereas binding to ssDNA was not (Fig. 1C). These results indicated that O-81 Ab cross-reacts with sHerp at the Ag binding site; the order of affinity of O-81 Ab is: ssDNA > > sHerp > dsDNA.

Next, to check the cross-reactivity of lupus polyclonal anti-dsDNA Abs to Herp, IgG anti-dsDNA Abs were purified from the sera of SLE patients using a protein A/G column and an immobilized dsDNA column. Patients with clinically apparent nephritis or low C3 titer were selected for the study. Lupus anti-dsDNA Abs showed a variety of binding activities to sHerp (Fig. 1D). Some lupus anti-dsDNA Abs (e.g., R29, S42, T48, T30, S40, Q30, T32, S57, T23) have been shown to bind to sHerp, whereas others (e.g., B53, P24, P4, P17, N31, and D55) showed little or no binding. Alternatively, Herp-reactive IgGs were similarly purified from the sera of SLE patients using a protein A/G column and an immobilized sHerp column. The anti-Herp Abs from SLE bound to dsDNA in most cases, although some others (e.g., P4, N31, O34) did not (Fig. 1E). Herp-reactive IgG could also be purified from normal sera, but these IgGs did not bind to ssDNA.

**Herp protein is expressed and caspsases are activated in the PBLs of patients with active SLE**

We examined Herp protein expression in the PBLs of patients with SLE by immunofluorescence microscopy with the HT2 mouse anti-Herp mAb (Fig. 2A). Patients with SLE were assigned to three groups according to disease activity: A, increase in SLE disease activity index (SLEDAI) ≥ 4 within 2 mo (onset or flare-up of SLE); B, SLEDAI ≥ 5 but increase of SLEDAI ≤ 3 within 2 mo (moderate disease activity without marked changes); C, SLEDAI ≤ 4 and increase of SLEDAI ≤ 3 within 2 mo (minimal disease activity without marked changes or remission). A and B were considered active SLE. Generally, many PBLs (+, ≥10 cells per high-power field [hpf]; ~500 cells) in group A showed strong expression of Herp and several PBLs (+, 1–9 cells per hpf) in group B were positive for Herp expression. In group C, several or no PBLs expressed Herp protein.

Herp protein was not expressed in the PBLs from healthy control subjects under normal conditions. Induction of Herp expression was observed when the PBLs were treated with thapsigargin, but the level of expression tapered off within the 72 h culture period (Fig. 2B). Alternatively, the PBLs from patients (Fig. 2, M.Sa. and M.N.) who developed SLE and had yet to receive treatment were positive for Herp protein, and its expression was still observed at the end of the 72 h culture period without stimulation (Fig. 2C). Activation of caspsases actually occurred in the Herp-positive PBLs of patients with active SLE (Fig. 3A). In the case of normal subjects, the expression of Herp and activation of caspsases occurred in the PBLs after treatment with thapsigargin (Fig. 3B). These results suggest that excessive endoplasmic reticulum (ER) stress followed by activation of caspsases occurs in the PBLs of patients in the active stage of SLE.

**Immunization with sHerp can elicit anti-DNA and anti-nuclear Abs, and induce glomerular IgG deposition in mice**

BALB/c mice were immunized with sHerp protein to investigate whether the immune response it induces can elicit anti-DNA Abs. After immunization with 100 μg sHerp and Ribi adjuvant R-700 (Corixa) twice and then with 50 μg sHerp alone once, anti-dsDNA Abs were elicited in sHerp-immunized mice (Fig. 4A). HEL, which is an exogenous Ag with strong antigenicity, was used as an...
immunization control. Anti-dsDNA Abs were observed in the sHerp-immunized mice, but not in those immunized with HEL (Fig. 4A). The nuclei of Hep2 cells were stained positively with the serum from sHerp-immunized mice on day 42, but not with those from HEL-immunized mice or untreated controls (Fig. 4C, left panel). Immunization with sHerp or HEL was continued every 2 wk until day 126, and there were no further significant changes in the titers of anti-dsDNA Abs in either group (Fig. 4A). The kidneys of the mice were examined by immunofluorescence microscopy with FITC-conjugated anti-mouse IgG on day 136. Glomerular IgG deposition was observed in the sHerp-immunized mice, but not in those immunized with HEL or in untreated controls (Fig. 4C, right panel). In the sHerp-immunized mice, however, there were no significant increases in urinary protein levels.

Next, to exclude the effects of small amounts of possible contaminants in the recombinant sHerp, BALB/c mice were similarly immunized with GST, the fusion partner of sHerp that was produced in the same way as sHerp, as a negative control. We used the oligonucleosomes as a positive control, because Rumore and Steinman (30) reported the presence of circulating oligonucleosomes in SLE plasma, and Voynova et al. (9) reported that anti-dsDNA Abs were elicited in BALB/c mice immunized with oligonucleosomes at a dose of 10 μg protein emulsified in Freund’s adjuvant three times. Because it has been reported that adjuvant itself can elicit autoantibody production in BALB/c mice (31), we immunized BALB/c mice with sHerp, GST, or oligonucleosomes three times without adjuvant. In contrast to our expectations, neither anti-dsDNA nor anti-oligonucleosome Abs were elicited in either GST-immunized or oligonucleosome-immunized mice.
mice, were measured by direct binding ELISA. Sera were diluted 1:300 in
immunized with GST, oligonucleosome, or sHerp, or in untreated 9-wk-old
tivities (closed bars) or anti-oligonucleosome activities (open bars) in mice


FIGURE 4. Response to Herp in mice. A, Anti-dsDNA activities in mice immunized with sHerp (closed bars) or HEL (open bars) were measured by direct binding ELISA. Sera were diluted 1:300 in PBS-T. Bound IgG was detected using HRP-conjugated goat F(ab’2) anti-mouse IgG-Fc Ab (Cappel). α, Mean titer in 10 untreated 25-wk-old female mice. ∆p < 0.05. B, Anti-Herp and anti-HEL activities in mice immunized with sHerp (closed bars) or HEL (open bars) were measured by direct binding ELISA. ∆p < 0.01. C, Anti-nuclear Abs in sHerp-immunized BALB/c mice. Anti-nuclear activity was evaluated by immunofluorescence staining of Hep-2 cells with serum from mice immunized with sHerp (day 42) or HEL (day 42) or from intact 14-wk-old mice (left panel). Glomerular deposition of IgG was examined in mice immunized with sHerp or HEL or in intact mice by immunofluorescence histochemistry (right panel). D, Anti-dsDNA activities (closed bars) or anti-oligonucleosome activities (open bars) in mice immunized with GST, oligonucleosome, or sHerp, or in untreated 9-wk-old mice, were measured by direct binding ELISA. Sera were diluted 1:300 in PBS-T; ∆p < 0.05 versus the untreated, GST, or oligonucleosome group.

Alternatively, anti-dsDNA Abs and anti-oligonucleosome Abs were elicited in sHerp-immunized mice (Fig. 4D).

A possible model of antigenic presentation of Herp in vivo

Next, we considered the in vivo conditions under which Herp is recognized by the immune system. ER stress is caused by a range of insults in daily life, such as chemical exposure, infection, and UV exposure. Cells exposed to excessive ER stress are induced to un-
dergo apoptotic cell death and express ER proteins on apoptotic blebs on the cell surface (32). Similarly, ER stress-induced apoptotic HeLa cells express Herp protein on the apoptotic blebs (Fig. 5A, Supplemental Fig. 1).

As EBV, which infects >90% of adults regardless of geographic location, has been suggested to play a role in the development of SLE (33), we focused on EBV as an example of viral infection. We postulated that ER stress might be increased when the virions of EBV are produced during lytic infection. EBV-transformed B cells were costained with HT2 anti-Herp Ab and anti-BZLF1 Ab after fixation and permeabilization, because BZLF1 protein initiates the switch from latent to lytic infection (34). Although most cells were negative for BZLF1 and Herp, a subpopulation of the cells coexpressed both molecules (Fig. 5B). Next, BALB/c mice were immunized with the membrane fraction of EBV-transformed B cells without adjuvant. Anti-Herp titer was significantly elevat-
ed in the immunized mice compared with intact controls (p = 0.016; Fig. 5C). Although the difference between the two groups in regard to mean anti-dsDNA Ab titer had a p value of 0.132, both anti-dsDNA Abs and IgG glomerular deposition were ob-
served in two of the immunized mice (Nos. 3 and 6; Fig. 5D, 5E).

Discussion

The results of this study indicate that the O-81 human anti-ss/dsDNA mAb and anti-dsDNA Abs, purified from the IgG of patients with SLE, cross-react with Herp. However, the binding magnitude for Herp was not correlated with that for dsDNA, and vice versa (e.g., A31, B53, and P14 [Fig. 1D, 1E]), probably owing to heterogeneity in polyclonal anti-dsDNA Abs of each patient with SLE. Moreover, Herp-binding IgG was detected in normal sera, but these IgGs did not bind to dsDNA. These observations indicate that Herp contains several different epitopes, some of which are responsible for binding to anti-dsDNA Abs.

Immunization with sHerp could elicit anti-dsDNA Abs in normal BALB/c mice (Fig. 4A), suggesting that Herp itself could be the Ag responsible for triggering anti-dsDNA Ab production in vivo. Singh et al. (35) reported that the inhibitory T cells in nonautoimmune mice limit autoantibody production. Immunization with Herp may overcome the limitation of autoantibody production by inhibitory T cells in nonautoimmune mice. Nucleosomes have been suggested as possible Ags responsible for triggering of anti-dsDNA Abs (9, 36). Mononucleosome-reactive Th clones augment the production of IgG autoantibodies to dsDNA, histones, and histone-DNA complex. However, immunization of SNF1 mice with pure mononucleosomes did not elicit production of IgG anti-dsDNA Abs (37). HMGB1-nucleosome complexes, but not HMGB1-free nucleosomes, induced anti-
dsDNA and anti-histone IgG responses in BALB/c mice (38). Polymavirus TAg-nucleosome complex, but not nucleosomes, can elicit histone-specific T cells in healthy individuals in vitro (39). In this study, neither anti-oligonucleosome Abs nor anti-
dsDNA Abs were elicited by immunization with oligonucleosome.

These data indicate that nucleosomes alone have poor antigenicity, but Ags that can bind to the nucleosomes render them immunogenic, similar to the hapten-carrier effect. Alternatively, Herp can elicit anti-dsDNA Abs and anti-oligonucleosome Abs (Fig. 4D). Similar to anti-dsDNA Abs, the mechanism responsible for production of anti-nucleosome Abs has not yet been clarified. It is possible that Herp is an Ag triggering production of both anti-
dsDNA Abs and anti-nucleosome Abs.

The reason why the epitopes on Herp are immunogenic is currently unclear. Herp, which is an intracellular protein, is scarcely expressed under normal conditions. Expression of Herp occurs in response to ER stress, and the expressed Herp is degraded rapidly
by polyubiquitination with Ube2g2/gp78 (40). Therefore, it is possible that immunotolerance against some epitopes on Herp is not perfect. Excessive ER stress induces not only strong expression of Herp but also apoptosis, and Herp is therefore presented on the surface of apoptotic blebs (Fig. 5A); this might allow recognition of Herp by the immune system even in healthy individuals (Fig. 1E). Naturally occurring Abs reacting with Herp in healthy individuals can aid in removing apoptotic cells by binding to Herp on apoptotic blebs. These Abs do not bind to DNA, whereas the sera from the sHerp-immunized mice did bind to DNA. This suggests that the epitopes on Herp mimicking DNA have low antigenicity, but become antigenic in the presence of a sufficient amount of Herp. Excessive expression of Herp over a longer period may result in recognition of the epitopes mimicking DNA by the immune system in vivo. The following observations in the current study should be noted. First, the PBLs from subjects in active SLE, especially at the time of onset or flare-up of the disease, tended to show Herp expression (Fig. 2A). Second, Herp was expressed for longer periods and at higher levels in the PBLs from patients with active SLE (Fig. 2A, 2C). Third, apoptotic PBLs expressing Herp are present in patients with active SLE (Fig. 3A).

**FIGURE 5.** Possible role of EBV-infected B cells in anti-DNA response in vivo. A, Herp protein was expressed on apoptotic blebs. B, Herp protein was coexpressed with BZLF1 in EBV-transformed B cells. C and D, Anti-Herp activities (C) or anti-dsDNA activities (D) in BALB/c mice immunized with the membrane fraction prepared from EBV-transformed cells. Left: The values of individual serum samples (day 63). Sera were diluted 1:300 in PBS-T. Right: The mean value for each group. *p < 0.05; **p > 0.05. E, Glomerular deposition of IgG was examined in the immunized mice (EBB Nos. 3 and 6) or untreated controls (untreated Nos. 2 and 3).
materials is impaired, the chance of exposure of Herp to the immune system may also increase. Indeed, such clearance deficiency has been reported in patients with SLE (41, 42). Our hypothesis is consistent with the report of Levine et al. (43), who succeeded in eliciting SLE-specific autoantibodies, including anti-DNA Abs, and in inducing overt glomerulonephritis in normal mice by immunization with apoptotic syngeneic thymocytes coupled with human β2-glycoprotein I and LPS.

Finally, we considered a practical model in which cell stress triggers an anti-DNA response via Herp in normal individuals. Natural infection with viruses can cause ER stress on a large scale in vivo (44). ER stress has been shown to increase when viral proteins are produced at high levels, such as in virion formation during the active lytic cycle of infection. EBV infection has been suggested to have a causative role in SLE (33). Patients with SLE have defective control of latent EBV infection (45); this might result in aberrant expression of BZLF1, a hallmark of EBV lytic infection, which has been detected in the PBLs of patients with SLE (46). In primary EBV infection, EBV infects tonsillar B cells in which lytic replication occurs, and differentiation of latently EBV-infected B cells into plasma cells in lymphoid tissues is associated with induction of the EBV lytic cycle (47). The expression of Herp is induced in cells entering the lytic phase of EBV infection and can be recognized by the immune system. In this study, we immunized BALB/c mice with the membrane fraction of EBV-transformed B cells, because Herp is an ER membrane protein. These immunized mice showed production of anti-Herp Abs (Fig. 5C). Moreover, anti-dsDNA Abs and glomerular IgG deposition were observed in two of these mice, but not in any of the untreated controls (Fig. 5D). These observations support the association between EBV and SLE.

Although immunization with sHerp can elicit anti-dsDNA Ab production and cause glomerular IgG deposition, neither a marked increase in urinary protein level nor overt nephritis were observed in sHerp-immunized mice despite immunization with sHerp many times (Fig. 4). Additional events may be necessary to increase the pathogenicity of Herp-induced anti-DNA Abs. Our findings indicate that immunization with sHerp can create conditions similar to silent lupus nephritis, which might be the earliest stage in the natural history of this disease (48). The results of the current study led to the following hypothesis: ER stress by common environmental factors (e.g., infection, chemicals, UV exposure) → Herp expression → recognition by the immune system of minor epitope(s) mimicking dsDNA → initial anti-dsDNA Ab production. Herp is a good candidate as a link between common environmental factors and the etiology of SLE.

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Disclosures
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References


