DNA hypomethylation is crucial for apoptotic DNA to induce systemic lupus erythematosus-like autoimmune disease in SLE-non-susceptible mice

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Objectives. Systemic lupus erythematosus (SLE) is characterized by serological presence of anti-double-stranded DNA (dsDNA) antibodies and its pathogenesis remains unclarified. Our previous work found that syngeneic activated lymphocyte-derived DNA (ALD-DNA) induced SLE-like autoimmune disease in the SLE-non-prone BALB/c mice. Here, the biological and chemical characteristics of the somatic DNA were focused upon to investigate their contribution to the autoimmunity induction to provide clues for the understanding of the pathogenesis of SLE in non-susceptible strains.

Methods. Induction of anti-dsDNA antibodies, glomerulonephritis and proteinuria was evaluated in BALB/c mice after subcutaneous immunization with apoptotic DNA (annexin-V +) extracted from concanavalin A or UV-treated apoptotic splenocytes or necrotic DNA from necrotic splenocytes. The hypomethylated apoptotic DNA and the normal DNA were then methylated and demethylated, respectively, by CpG methylase or 5-azacytidine treatment to re-evaluate their immunogenicity in BALB/c mice.

Results. It was apoptotic but not necrotic DNA that induced SLE-like autoimmune disease and the level of apoptotic DNA was associated with the level of anti-dsDNA antibodies. The apoptotic DNA exhibited significantly lower methylation levels than the normal DNA. Methylation of the hypomethylated apoptotic DNA significantly impaired its ability to induce anti-dsDNA antibodies and proteinuria, while demethylation of the normal or necrotic DNA endowed them with the immunogenicity to induce the SLE-like syndrome.

Conclusions. Our study provides direct evidence showing that DNA hypomethylation is essential for apoptotic DNA to induce SLE-like autoimmune disease in non-susceptible mice, which may help in elucidating the pathogenesis of SLE.

Key words: Systemic lupus erythematosus, Anti-dsDNA antibody, Apoptosis, Hypomethylation.

Introduction

Systemic lupus erythematosus (SLE) is a heterogeneous disease manifested by the presence of anti-double-stranded DNA (dsDNA) antibodies (Abs), which is closely correlated with the clinical syndrome and hence of diagnostic and even prognostic value [1–4]. The diversity of clinical manifestations and disease phenotypes, together with the limited accessibility of the human tissues and samples, has hindered the study of human SLE. Although the presently available animal models such as NZB, NZM, (NZB × NZW)F1, (SWR/NZB)F1, MRL/lpr, C3H/gld/gld and BXSB mice have allowed us to make meaningful progress towards understanding the pathogenesis of SLE [5], each of these murine models carries a unique set of susceptibility genes, defined or ambiguous, which would to some extent limit our understanding of the pathogenesis of SLE. From this point of view, establishment of a murine lupus model with normal gene background could be very meaningful for the study of SLE pathogenesis.

Our previous study demonstrated that if we stimulated syngeneic lymphocytes by concanavalin A (ConA) for 2–3 days, and then extracted DNA from the lymphocytes to immunize non-susceptible BALB/c mice, this so-called activated lymphocyte-derived DNA (ALD-DNA) was capable of inducing an autoimmune disease that closely resembled human SLE including high levels of anti-dsDNA Abs, glomerulonephritis and proteinuria [6]. This murine SLE model therefore provides a useful tool for us to continue the study on the immunogen and pathogenesis of SLE [7]. The previous study indicated that only DNA extracted from the ConA-incubated, excessively activated lymphocytes, but not resting lymphocytes, could acquire the immunogenicity to induce anti-dsDNA Abs. ConA activation usually leads to the apoptosis or necrosis of lymphocytes. So the next question is which DNA, ‘apoptotic DNA’ or ‘necrotic DNA’, would be responsible for the induction of SLE-like syndrome? And which biochemical properties of the DNA would influence its immunogenicity?

Accumulating evidence has indicated that the apoptotic DNA might be related to the pathogenesis of SLE [8, 9]. A defect in the clearance of apoptotic cells has been observed in SLE patients [10, 11], which results in the accumulation of apoptotic cells and the subsequent release of the cellular contents normally shielded from the adaptive immune system [12]. Moreover, DNA purified from the serological DNA–anti-DNA autoantibody complexes of SLE patients has an average length consistent with the apoptotic cleavage of chromatin [13]. In addition, production of IFN-β in DNase II–null mice suggested that endogenous DNA that escaped apoptotic DNA fragmentation could activate immune responses [14]. However, there is still a lack of direct evidence for the immunogenicity of apoptotic DNA to induce anti-dsDNA Abs in the murine models.

To test our hypothesis that the apoptotic DNA was capable of inducing SLE-like syndrome in SLE-non-prone mice, BALB/c mice were immunized with syngeneic apoptotic DNA, necrotic DNA or normal DNA derived from apoptotic, necrotic or resting splenocytes. We found that only apoptotic DNA induced high levels of anti-dsDNA Abs, glomerulonephritis and proteinuria. We then focused our attention on the methylation level of the apoptotic DNA and demonstrated that hypomethylation was crucial for apoptotic DNA to induce SLE-like autoimmune disease in SLE-non-susceptible mice.

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Materials and methods

Mice
Female BALB/c mice between 6 and 8 weeks of age were purchased from the Center of Experimental Animals of Fudan University and housed in a pathogen-free mouse colony at our institution. All animal experiments were performed according to the Guide for the Care and Use of Medical Laboratory Animals (Ministry of Health, PR China, 1998) and with the ethical approval of the Shanghai Medical Laboratory Animal Care and Use Committee as well as the Ethical Committee of Fudan University.

Preparation and manipulation of the splenocytes
Spleens of naïve BALB/c mice were aseptically removed and teased on a nylon mesh immersed in chilled RPMI-1640 medium (Gibco, USA) in a plastic dish. Cells that passed through a nylon mesh were washed twice with RPMI-1640. The erythrocytes were lysed with Tris–NH₄Cl and the remaining splenocytes were diluted to a final concentration of 2 × 10⁶ cells/ml and cultured in RPMI 1640 containing 10% heat-inactivated fetal bovine serum (Gibco), 2 mmol/l glutamine (Sigma, USA), 100 IU/ml penicillin and 100 μg/ml streptomycin. The activated splenocytes were prepared by incubation with 5 μg/ml ConA (Sigma) for 48 h. Apoptotic splenocytes were prepared by ConA stimulation or by UV irradiation (200 mJ/cm²) followed by 3 h culturing at 37°C, as previously described [12]. The apoptotic cells were stained by annexin-V-fluorescein isothiocyanate (FITC) (BD Biosciences, USA) and sorted by flow cytometry (BD Biosciences). Necrotic splenocytes were generated by freezing (−80°C, 12 min) and thawing (37°C) and confirmed through light microscopy after trypan blue staining, as previously described [12].

DNA extraction and purification
According to our previously described method [5], genomic DNA was extracted from apoptotic, necrotic and normal (resting) splenocytes using the UltraPure™ genomic DNA purification kit, as recommended by the manufacturer (SBS Genetech, Shanghai, China), and treated with S1 nuclease (TaKaRa, Japan) and proteinase K (Sigma) according to the manufacturer’s instructions and then purified again. The concentration of DNA was determined by the absorbance (A) at 260 nm. The final A260/A280 for all the DNA preparations was more than 1.8. Less than 0.01 U/μg endotoxin was present in any of the DNA samples, based on a Limulus amoebocyte lysate assay (BioWhittaker, Walkersville, MD, USA).

Mice immunization with DNA
BALB/c mice were divided into four groups (n = 8) and subcutaneously injected under the dorsal skin with 0.2 ml of an emulsion containing 50 μg of ALD-DNA, normal DNA, apoptotic or necrotic DNA [dissolved in phosphate-buffered saline (PBS)] plus complete Freund’s adjuvant (CFA; Sigma). Mice receiving an equal volume of PBS plus CFA were used as controls. Mice were given two booster immunizations consisting of 50 μg DNA emulsified with incomplete Freund’s adjuvant, 2 and 4 weeks later. Serum and urine samples were collected every 2 weeks.

ELISA measurement of serum anti-dsDNA IgG
Serum anti-dsDNA immunoglobulin G (IgG) Abs and their isotypes were detected as previously described [6]. Briefly, protamine sulphate pre-treated 96-well microtitre plates (Nunc, Denmark) were coated with 50 μg/ml calf thymus dsDNA (Sigma) for 2 h at 37°C and then placed overnight at 4°C. After washing three times with PBS containing 0.05% Tween-20 (PBST), the plates were serially diluted (in PBST containing 10% calf serum and 5% goat serum) serum samples were added and incubated for 2 h at 37°C then with horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG, IgG1, IgG2a, IgG2b and IgG3 (Southern Biotech, USA). After colour development by O-phenylenediamine (OPD, Sigma), the reaction was stopped by 2 NH₄SO₄ and absorbance at 492 nm was measured in a microplate reader (BioLab, USA).

Analysis of the antibody affinity of the anti-dsDNA IgG
The affinity of the anti-dsDNA Abs was evaluated using diethylamine (DEA)-inhibitory anti-dsDNA Abs ELISA [15]. This is based on the ability of the mild chaotropic agent DEA (Sigma) to inversely inhibit antibody binding in proportion to Ab affinity. The serum was incubated together with 10 mmol/l of DEA (based on initial titration experiments); again, all sera from a series were assayed on the same plate. Optical density (OD) was plotted against log10 of serum dilution (dilution factor) and the leftward shift in the dose–response curve in the presence of DEA was measured at 50% of the maximum OD. Results were expressed as the reciprocal log10 of the shift and this value (inhibition index) was taken as a measure of the functional affinity.

Evaluation of the renal pathology
Eight weeks after the first DNA immunization, histopathology assessment was done on 4 μm sections of paraffin-embedded, formalin-fixed kidneys stained with haematoxylin and eosin. Glomerulonephritis was evaluated using light microscopy by two pathologists who were blinded to the study. Cryosections (4 μm) of kidneys of mice were incubated with FITC-conjugated goat anti-mouse IgG (Sigma) for the analysis of renal immunoglobulin deposits. Images were collected using a fluorescence microscope (Nikon, Japan). The elution of immunoglobulin from isolated glomeruli was performed by 2 h incubation with PBS (pH = 6.9) containing 5 U/ml DNase 1 and 0.002 mol/l MgSO₄. After centrifugation, the supernatant was obtained and dialysed against PBS (pH = 7.2) containing 0.001 mol/l EDTA overnight and then assayed for the presence of anti-dsDNA IgG by ELISA.

ELISA measurement of the proteinuria
Proteinuria was measured with the Bradford assay using a commercially available kit (GeneRay Biotech, Shanghai, China), according to the manufacturer’s specification.

Analysis of the methylation level of DNA
The methylation level of DNA was analysed by dot-blot analysis, as described previously [16]. Briefly, DNA solution was dotted onto a Hybond nitrocellulose membrane (Amersham, Arlington Heights, IL, USA). The membrane was then probed with mouse anti-5-methylcytosine (5-MeC; Calbiochem, USA) mAb, washed with T-BST (Tris-buffered saline plus Tween-20, pH = 7.6) and incubated with 1:2000 dilution of HRP-conjugated anti-mouse-IgG (Southern Biotech) for 1 h. The membrane was then treated with enhanced chemiluminescence western blotting detection reagent (Amersham) and exposed to Kodak autoradiograph films. The level of the methylated DNA was measured by a Scion Image Analysis System (Scion Corp., Frederick, MD, USA). Equal loading of the DNA was indicated by equal intensity of 0.02% methylene blue staining.

Methylation of the apoptotic DNA
DNA methylation was achieved by incubation of DNA with CpG methylase M.SssI (New England Biolabs, Beijing, China) in an 80 μl reaction solution containing 8 μl of 10 x NE Buffer, 0.4 μl of Hypomethylated apoptotic DNA induces SLE in non-prone mice
32 mmol/l SAM, 4 μl of M.SssI and 80 μg of the apoptotic DNA for 1 h at 37°C, as previously described [17].

Demethylation of normal DNA and necrotic DNA
To make demethylated DNA, normal splenocytes or frozen-thawed splenocytes were treated with 5-azacytidine (Sigma) at a concentration of 4 μmol/l for 3 days to achieve efficient demethylation, as previously described [18]. Then the demethylated normal DNA and necrotic DNA were prepared.

Statistical analysis
Statistical significance was assessed using Student's t-test or Mann–Whitney U-test unless otherwise noted, and data are given as mean ± s.d. unless otherwise noted. Statistical analyses of data were performed using the GraphPad Prism (version 4.0) statistical program. P < 0.05 was taken as significant.

Results
Apoptotic DNA extracted from the activated lymphocytes was responsible for the induction of anti-dsDNA antibodies in the non-autoimmune BALB/c mice
Our previous study [6] has shown that the ALD-DNA, but not resting lymphocyte-derived DNA (normal DNA), could induce high levels of anti-dsDNA Abs after subcutaneous immunization in BALB/c mice (Fig. 1A). Since the ALD-DNA was extracted from the syngeneic splenocytes pre-activated by ConA for 2-3 days, we wanted to see if the ConA incubation time would influence the immunogenicity of the ALD-DNA. Interestingly, if we prolonged the ConA-lymphocyte incubation time before the DNA extraction, it was found that the anti-dsDNA levels induced by DNA immunization increased as the ConA incubation time extended, and the elevated Ab levels were associated with the prolonged incubation time from day 0 to day 6 (P < 0.05, Fig. 1B). Then the apoptotic level of variously ConA-incubated splenocytes was analysed and an increased apoptotic level of the splenocytes was found, which was only 4.56% on day 0 but increased to 85.17% on day 6 (Fig. 1C). These data suggested that the apoptotic lymphocyte-derived apoptotic DNA might be responsible for the induction of anti-dsDNA Abs in BALB/c mice.

To confirm the above hypothesis, the apoptotic DNA was extracted form the annexin-V+ cells from the ConA-stimulated splenocytes by FACS cytometry, then used to immunize BALB/c mice, using the non-apoptotic DNA ( annexin-V- cells) as the negative control. It was revealed that only the apoptotic DNA but not the non-apoptotic DNA induced high levels of anti-dsDNA Abs, in a dose-dependent manner, showing that the minimal dose for the apoptotic DNA to induce anti-dsDNA Abs would be not less than 5 μg for each immunization (Fig. 1D and E).

To exclude the possible interfering effect of necrotic DNA mixed in the ALD-DNA on the induction of anti-dsDNA Abs, necrotic DNA was also extracted from the frozen-thawed necrotic splenocytes and then used to immunize mice. However, the necrotic DNA failed to induce anti-dsDNA Abs compared with the apoptotic DNA (Fig. 1F), suggesting that the apoptotic DNA but not necrotic DNA extracted from the activated lymphocytes was really responsible for the generation of anti-dsDNA Abs in the non-autoimmune mice.

Lymphocyte activation course was not required for the apoptotic DNA to induce anti-dsDNA antibodies
The apoptosis of lymphocytes could be induced through the activation-induced cell death (AICD) pathway or merely physicochemical reagent treatment. To clarify whether ConA-mediated non-specific activation course was required for the apoptotic DNA to induce anti-dsDNA Abs, apoptotic splenocytes with 83.27% apoptotic level were also generated by UV irradiation (Fig. 2A). It was found that the apoptotic DNA derived from UV irradiation-induced apoptotic splenocytes [apoptotic DNA (UV)] also induced high levels of anti-dsDNA Abs comparable with those induced by apoptotic DNA derived from ConA activation-induced apoptotic splenocytes [apoptotic DNA (AICD)] (Fig. 2B). There was almost no significant difference in the affinity or the IgG isotypes of the anti-dsDNA Abs between the apoptotic DNA (AICD) and apoptotic DNA (UV)-treated mice (Fig. 2C and D). Comparable levels of anti-dsDNA IgG1, IgG2b and IgG3 were induced by the two kinds of apoptotic DNA except that more IgG2a was generated in the apoptotic DNA (AICD)-treated mice (P < 0.05, Fig. 2D). Finally, a similar dose-dependent manner for the generation of anti-dsDNA antibodies was also found after apoptotic DNA (UV) immunization (Fig. 2E). These results indicated that the activation of lymphocytes by ConA was not required for lymphocyte-derived apoptotic DNA to induce anti-dsDNA antibodies.

Mice immunized with apoptotic DNA manifested SLE-like autoimmune disease, as evidenced by glomerulonephritis, immunoglobulin deposition and proteinuria
To directly assess whether non-autoimmune mice could develop autoimmune disease after immunization with the annexin-V+ apoptotic DNA, 4 weeks after the three DNA injections, kidney tissues were isolated from mice and histopathological analysis was performed. Compared with the normal or necrotic DNA-immunized mice in which the tissue infiltration of leucocytes and glomerulonephritis was absent (Fig. 3Ac,d), we found seven of eight apoptotic DNA (ACID or UV)-immunized mice showed glomerular hypercellularity and tubular epithelial cell swelling and necrosis (Fig. 3Ab,c). Immunohistological analysis on kidney sections of the apoptotic DNA-immunized mice revealed severe glomerular deposits of IgG antibodies when compared with those of necrotic or normal DNA-immunized mice (Fig. 3B). Furthermore, IgG antibodies eluted from murine nephritic kidneys recognized apoptotic DNA, but not necrotic or normal DNA (P < 0.01, Fig. 3C), indicating they are specific against apoptotic DNA and may form immune complexes with apoptotic DNA in the body that may contribute to the renal IgG deposits. Finally, a high level of urine proteins was detected only in the apoptotic DNA-treated mice, especially in the apoptotic DNA (AICD)-treated mice (P < 0.05, Fig. 3D). These results suggested that the apoptotic DNA-immunized mice developed SLE-like autoimmune disorders.

Hypomethylation was crucial for the apoptotic DNA to induce anti-dsDNA antibodies and proteinuria
Accumulating studies have indicated the potential role of DNA hypomethylation in the pathogenesis of autoimmune diseases [18-21]. We next sought to determine whether the methylation level of the apoptotic DNA was changed and whether it would influence the immunogenicity of the apoptotic DNA. First, a dot-blot analysis revealed significantly lower levels of 5-MeC of the up-methylated apoptotic DNA to induce the anti-dsDNA Abs compared with those of necrotic or normal DNA (P < 0.05, Fig. 4A). To directly explore whether the hypomethylation of apoptotic DNA was crucial for the induction of the anti-dsDNA responses, we significantly elevated the methylation level of the apoptotic DNA by methylase treatment (P < 0.05, Fig. 4A) and used it to immunize BALB/c mice. We found that the ability of the up-methylated apoptotic DNA to induce the anti-dsDNA Abs and proteinuria was robustly impaired compared with that of the untreated apoptotic DNA (P < 0.05, Fig. 4B and C). Surprisingly, if we reduced the methylation level of the necrotic
or normal DNA by the treatment of splenocytes with 5-aza-cytidine before DNA extraction (Fig. 4A), the demethylated necrotic or normal DNA then acquired the ability to generate significantly higher levels of anti-dsDNA Abs and proteinuria than their primary counterparts ($P < 0.05$, Fig. 4B and C), which reached at least 60–70% of levels as generated by the apoptotic DNA immunization. These data suggested that the degree of hypomethylation of the apoptotic DNA could determine the amount of anti-dsDNA Abs and the severity of SLE-like autoimmune disease.

Fig. 1. Induction of anti-dsDNA Abs in BALB/c mice immunized with syngeneic apoptotic or necrotic DNA. (A and B) The levels of the anti-dsDNA Abs in mice immunized with 50μg of the ALD-DNA (ConA-ALD-DNA) or normal DNA (resting splenocyte-derived DNA). (C) The apoptotic level of the ConA-stimulated syngeneic splenocytes was assayed by annexin V staining at the indicated time. (D) Generation of the anti-dsDNA Abs in mice immunized with 50μg of the apo DNA (apoptotic/annexin V− splenocyte-derived DNA) or non-apo DNA (resting/annexin-V− splenocyte-derived DNA). (E) The dose-dependent effect of the apoptotic DNA to induce the anti-dsDNA Abs. (F) Anti-dsDNA Abs induction by immunization with 50μg of necDNA (necrotic splenocyte-derived DNA) or apoDNA (apoptotic splenocyte-derived DNA). All data shown are representative of at least three independent experiments. Data represent the mean values and s.d. for eight mice in each group.
Discussion

Multiple genetic factors such as intrinsic immunological abnormalities, sex hormones and environmental factors such as infectious agents contribute to the development of SLE in humans. Although various lupus experimental murine models carry various sets of susceptibility genes predisposing to autoimmunity and only possess one or some of the features of human SLE, they may enable investigators to address the relevance of each specific contributory agent and altogether shed light on the pathogenesis of SLE. Our SLE murine model, established from normal BALB/c strains by immunization with a defined autoantigen (dsDNA derived from apoptotic splenocytes), represents an ideal platform for the further investigation of the immunogenicity of mammalian DNA and the pathogenesis of SLE in the SLE non-susceptible strains.

What triggered us to start the previous and present study are some interesting findings from the study of human SLE. As we have known, the anti-dsDNA Ab represents the formal SLE classification criterion in human SLE diagnosis. Several human studies have revealed that substantial amounts of DNA fragments circulating in the plasma of SLE patients and associated with the disease activity [22]. Another interesting finding is that human lupus antibody--DNA immune complexes purified from active SLE patients stimulated dendritic cells (DCs) to produce cytokines and chemokines [23]; DNA fragments separated from immune complexes (IC) showed high frequencies of CpG dinucleotides while the anti-dsDNA antibodies recognized some unique (G+C)-rich DNA sequences. Meanwhile, the methylation level of the circulating DNA fragments seems to have decreased. Since unmethylated CpG motifs display an immune-stimulatory effect [24], synthetic oligonucleotides based on the DNA sequences purified from the SLE patients' blood could stimulate human peripheral blood mononuclear cells to generate IL-12 and IFN-γ [25]. All these human relevant data suggest that releasing of some amounts of stimulatory autologous DNA into the circulation of SLE patients might be responsible for the induction of anti-dsDNA Abs and could possibly induce direct cellular activation and may be related to disease progression. Since direct evidence from human experiments is inaccessible at present, we set out to test this hypothesis using our non-susceptible BALB/c SLE model.
Here we demonstrated that the apoptotic syngeneic DNA but not necrotic DNA actively induced anti-dsDNA Abs and SLE-like autoimmune disease in BALB/c mice. In fact, the release of the usually sequestered DNA by apoptotic cells seems to be a fundamental step in SLE induction, which has been indicated in mice with impaired apoptotic cell clearance, DNase II deficiency or complement C4 deficiency [9, 26, 27]. Our data extended these findings by providing direct evidence demonstrating that the syngeneic apoptotic DNA might be the immunogen of SLE. We found that the apoptosis-inducing course would not influence the immunogenicity of the apoptotic DNA (AICD- or UV-derived) except that a significantly elevated anti-dsDNA IgG2a level was induced by apoptotic DNA (AICD). Meanwhile, there seemed a close correlation between the enhanced IgG2a level and the increased urine protein levels seen in the apoptotic DNA (AICD)-immunized mice, indicating a possible Th1 immune bias that could be related to a more severe renal pathology. This result is consistent with the previous findings that the Th1-type immune response may play a pivotal role in SLE pathogenesis [12, 28].

Our findings showed that the hypomethylation of apoptotic DNA was crucial for the induction of SLE-like autoimmune disease. Actually, the relationship between DNA methylation and SLE has long been under investigation. Methylation of DNA is an important regulatory means to suppress the transcription of certain genes [29, 30]. Differences in the extent of methylation exist among specific genes or organs in both murine and human SLE to exert the regulatory function. DNA hypomethylation might be critical for the development of SLE because: first, SLE-like autoimmunity could be induced by hypomethylated CpG motifs and such motifs were detected in serum from SLE patients [31, 32]; second, SLE lymphocytes (especially T cells) show hypomethylation and reduced expression of DNA methyltransferase 1 (DNMT-1) mRNA [33–35]; and third, even treatment with demethylating agents such as 5-azacytidine, procainamide and hydralazine could induce SLE-like autoimmunity [36–38]. Here we provided direct evidence to demonstrate the crucial role of DNA hypomethylation in the induction of SLE by showing that up-regulation of hypomethylated apoptotic DNA significantly attenuated its immunogenicity to induce the anti-dsDNA Abs and proteinuria, while the demethylated necrotic or normal DNA acquired the ability to induce SLE-like syndrome. However, the methylase treatment of the apoptotic DNA failed to completely block its immunogenicity, suggesting that the hypomethylation level was a crucial but not an exclusive factor responsible for the apoptotic DNA to induce the SLE-like autoimmune disease.

The hypomethylated apoptotic DNA could also be considered as an immune-regulatory DNA. The regulatory role of DNA on the immune system was not clarified until the discovery of the CpG motif. The naturally existing bacterial-derived unmethylated CG dinucleotides containing certain CpG motifs were found to possess a very potent stimulatory effect on the human innate

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**Fig. 3.** The renal pathology of apoptotic-DNA-immunized mice, as evidenced by glomerulonephritis, immunoglobulin deposits and proteinuria. (A) Four weeks after three DNA immunizations, glomerulonephritis in the kidney sections is shown by haematoxylin and eosin staining from mice immunized with apoptotic DNA (AICD) (a, 200×), apoptotic DNA (UV) (b, 200×), necrotic DNA (c, 200×) and normal DNA (d, 200×). Results are representative of eight mice. (B) Kidney cryosections were also immunofluorescently stained with anti-mouse IgG conjugated with FITC. Shown are glomeruli with IgG (green) antibody deposits (100×). (C) The IgG elution from kidneys of mice receiving apoptotic DNA was able to recognize apoptotic DNA, as evidenced by ELISA. (D) The urine protein level in the urine samples of mice was measured by the Bradford assay. Results are representative of three independent experiments. **P<0.01.
However, the stimulatory hypomethylated apoptotic DNA generated in the present study is a totally different DNA from the aforementioned CpG ODN, although both exhibited potent immune-stimulatory effects. It comprises total genomic DNA, not a short sequence of unique DNA motifs, and most importantly it actively induces the anti-dsDNA Abs after immunization, indicating its specific immune-stimulatory effects.

To conclude, this study is an extension of our previous work in that it directly demonstrates that syngeneic hypomethylated apoptotic DNA is able to induce SLE-like autoimmune disease in SLE non-susceptible BALB/c mice. These findings broaden our understanding of the pathogenesis of SLE and the relevance of this mechanism in relation to the pathogenesis of human SLE needs further investigation.

Rheumatology key messages

- Immunization with apoptotic DNA induced SLE-like autoimmune disease in non-susceptible BALB/c mice.
- Hypomethylation is crucial for apoptotic DNA to induce SLE-like disease.

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References

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