

## A potential trigger of nephritogenic anti-DNA antibodies in lupus nephritis

### ABSTRACT

Anti-DNA antibodies play an essential role in the pathogenesis of lupus nephritis; however, mammalian DNA alone, however, is poorly immunogenic. We speculated that a non-DNA substance is the antigenic trigger for the production of human nephritogenic anti-DNA antibodies is a non-DNA substance. The cDNA library from the peripheral blood lymphocytes (PBLs) of a patient with active lupus nephritis was screened using the single-chain Fv of a human monoclonal nephritogenic O-81 anti-DNA antibody in a two-hybrid system. A clone containing the gene of an endoplasmic reticulum (ER) stress-inducible protein, Herp, was obtained. The O-81 antibody bound to recombinant Herp protein was synthesized by *Escherichia coli*. Immunization of BALB/c mice with Herp elicited the production of both anti-double-stranded DNA (anti-dsDNA) and anti-single-stranded DNA (anti-ssDNA) antibodies in BALB/c mice and formed resulted in deposits of IgG deposits in renal glomeruli. Anti-DNA antibodies purified from systemic lupus erythematosus (SLE) sera bound to Herp. Moreover, anti-Herp antibodies showed specific binding to DNA. Herp was spontaneously expressed in the PBLs of patients with active SLE, but not in the PBLs of healthy subjects. These results imply suggests that an inducible intracellular self-protein represents a candidate trigger for the production of human nephritogenic anti-DNA autoantibodies. Any cell stress causing ER stress—such as viral infection, ultraviolet radiation, and chemicals—might be responsible for anti-DNA antibody production via Herp.

**Comment [B1]:** Should “chemicals” be “toxins”?

### INTRODUCTION

In systemic lupus erythematosus (SLE), anti-DNA antibodies show not only both high incidence, but also and high disease specificity, and play an essential role in the pathogenesis of lupus nephritis.<sup>1</sup> The production of anti-DNA antibodies is a known representative distinguishing feature of SLE at the molecular level, and the initiation of anti-DNA antibody production may be a key point in the commitment of SLE. However, mammalian DNA alone, however, is poorly immunogenic.<sup>2</sup> The trigger for anti-DNA antibody production thus remains enigmatic, particularly in humans.

One possible mechanism for anti-DNA antibody production is a hapten-carrier relationship in which DNA is the hapten and the DNA-binding substance is the carrier. Anti-DNA response in mice could be evoked by immunization of double-stranded DNA (dsDNA) with either the 27-amino acid nucleic acid-binding Fus1 peptide,<sup>3</sup> or with polyoma BK virus large T antigen,<sup>4</sup> in mice. DNase I-dsDNA

**Comment [B2]:** Should “commitment” be “commencement” or “onset,” or would you like to imply a later stage of the disease? And is “and the initiation of anti-DNA antibody production may be a key point in the commitment of SLE” redundant with the previous sentence, “In systemic lupus erythematosus (SLE), anti-DNA antibodies ... play an essential role in the pathogenesis of lupus nephritis”?

complex,<sup>5</sup> nucleosomes, ~~or~~and crude histone<sup>6</sup> are also immunogenic, causing the production of IgG anti-DNA antibodies. This~~ese~~ indicates a possible role for DNA-protein complexes to break immune tolerance ~~of~~ DNA.

Another possible mechanism is molecular mimicry. Some anti-DNA antibodies can cross-react with non-nucleic acid self-antigens, such as cytoskeletal proteins,<sup>7</sup> extracellular matrix protein HP8,<sup>8</sup> brush border myosin,<sup>9</sup> calreticulin,<sup>10</sup> NR2 glutamate receptor,<sup>11</sup> or  $\alpha$ -actinin.<sup>12-14</sup> ~~Diamond et al.~~ reported that immunization with the peptide DWEYSVWLSN, which is recognized by mouse monoclonal anti-dsDNA antibody, elicited the production of anti-dsDNA antibodies in normal mice.<sup>15</sup> ~~Although~~ ~~Th~~is indicates that a non-nucleic acid antigen can elicit anti-DNA antibodies in normal animals. ~~However~~, no proteins containing this exact peptide sequence have been reported. ~~Thus~~, no molecule mimicking DNA that can elicit anti-DNA antibodies has yet been identified, especially in humans. ~~The Our~~ aim of this ~~study~~ was to elucidate the antigenic trigger for production of human nephritogenic anti-DNA antibodies.

**Comment [B3]:** Diamond, et al. has not been included in the reference list.

**Comment [B4]:** The meaning of this sentence is not clear. No such molecule has been identified at all, or no such molecule has been identified in humans? One possible rewrite: Thus, no molecule mimicking DNA that can elicit anti-DNA antibodies has yet been identified in humans. [And perhaps add something about the status of the identification of such a molecule in other species if relevant.] Or, e.g., Thus, no molecule mimicking DNA that can elicit anti-DNA antibodies has yet been described: identification of such a molecule in humans is particularly important. [And perhaps add why this is important.]

## METHODS AND RESULTS

To search for the antigenic trigger of anti-DNA antibody production, we adopted a human nephritogenic anti-DNA antibody, O-81, as a probe.<sup>16</sup> The cDNA library from the peripheral blood lymphocytes (PBLs) of a patient with active lupus nephritis was introduced into the target vector of a bacterial two-hybrid system. The single-chain Fv of the O-81 antibody (~~O-81SeFv~~) was ~~made-produced~~ from the O81VH and O81Vk genes and inserted into the bait vector.<sup>17</sup> ~~As a result of screening, a~~ clone containing the gene of an endoplasmic reticulum (ER) stress-inducible protein, Herp, was obtained. O-81 antibody specifically bound to recombinant Herp protein ~~was~~ synthesized in a bacterial expression system.

Each 6-week-old ~~female~~ BALB/c-~~female~~ mouse was immunized intraperitoneally with Herp. Immunization with Herp elicited both anti-dsDNA and anti-single-stranded DNA (anti-ssDNA) antibodies in BALB/c mice (~~Table 1~~) and ~~formed-resulted in~~ deposits of IgG in renal glomeruli. These phenomena were not observed in control mice.

**Comment [B5]:** A table may not be necessary for the presentation of these results. You might wish to incorporate the information from Table 1 into the text here.

Anti-DNA antibodies were obtained from the sera of patients with active lupus nephritis using a DNA-immobilized sepharose. ~~Not only~~Both O-81 antibody, ~~but also~~ and purified anti-DNA antibodies bound to recombinant Herp protein. ~~Binding of anti-DNA antibodies was~~ ~~interfered-with~~impeded by Herp, ssDNA, or dsDNA. ~~This indicates~~ ~~suggesting~~ that parts of the anti-DNA antibodies crossreact with Herp.

**Comment [B6]:** I attempted to clarify this sentence—please confirm that the sentence is accurate as edited. Is it correct that the binding of the antibodies to Herp is impeded by Herp or dsDNA or ssDNA?

To confirm this, anti-Herp antibodies were purified from the sera of patients with SLE using Herp-immobilized sepharose. Purified anti-Herp antibodies bound to both dsDNA and ssDNA. Autoantibodies crossreacting with DNA and Herp thus exist in patients with SLE.

Indirect immunofluorescence microscopy using monoclonal anti-Herp antibody demonstrated that

Herp protein was spontaneously expressed in the PBLs of patients with active SLE, but not in the PBLs of healthy subjects. Expression of Herp in the PBLs ~~from~~of SLE patients was observed *in vitro* for up to 72 hours without any stimulation ~~in vitro~~.

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**Comment [B7]:** I attempted to clarify this sentence—please confirm that the sentence is still accurate as edited. The observation of Herp expression in PBLs occurred in vitro?

## CONCLUSIONS

Our results suggest that crossreactive anti-DNA and anti-Herp antibodies exist in patients with SLE. Immunization ~~with~~of Herp can elicit production of anti-dsDNA ~~as well as~~and anti-ssDNA antibodies in normal mice. ~~There may be a~~An antigenic epitope(s) on the Herp protein may mimicking DNA ~~on Herp protein~~. ~~Because~~As Herp is an inducible intracellular protein and expression is usually transient, immune tolerance ~~of~~te all epitopes on Herp may not be complete. When lymphocytes that overexpress Herp protein undergo either apoptosis or necrosis, we speculate that Herp protein might be exposed on the apoptotic bleb or released from necrotic cells, ~~and be recognized by the immune system~~. When the epitope mimicking DNA on Herp protein is recognized by the immune system, crossreactive anti-Herp and anti-DNA antibodies could be produced. Cell stress (e.g., infection, chemicals, or ultraviolet radiation, ~~etc.~~) accompanying ER stress may possibly increase the risk of anti-DNA antibody production via induction of Herp (Fig. 1). Further investigations are needed to clarify the antigenicity and overexpression of Herp in patients with SLE.

**Comment [B8]:** Should “chemicals” be “toxins”?

**Comment [B9]:** Please provide a figure caption for Figure 1.

## REFERENCES

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Table 1: Anti-dsDNA or Anti-ssDNA activity of sera (OD405)

	Herp-immunized Mice	Control Mice	t-test
dsDNA	0.364 ± 0.066	0.051 ± 0.012	p < 0.01
ssDNA	2.569 ± 0.190	0.376 ± 0.019	p < 0.001

**Comment [B10]:** Please define OD405.

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