Specific modulation of the anti-DNA autoantibody–nucleic acids interaction by the high affinity RNA aptamer

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Abstract

Anti-DNA autoantibodies are one of the frequently found autoantibodies in systemic lupus erythematosus patient sera. RNA aptamers for the monoclonal G6-9 anti-DNA autoantibody were selected from a random pool of RNA library. Binding affinity of the best aptamer is around 2 nM, which is at least 100-fold higher than that of cognate DNA antigen to the autoantibody. Aptamer binds specifically to the G6-9 autoantibody but not to other similar autoantibodies. Minimal binding motif of the aptamer was mapped, providing a hint for a natural epitope of the autoantibody. DNA binding to the G6-9 autoantibody is shown to be efficiently inhibited by the aptamer. Such binding property of the RNA aptamer could be used not only as a modulator for the pathogenic anti-DNA autoantibody, but also as a useful biochemical reagent for elucidating a fine specificity of the autoantibody–nucleic acid interaction.

Keywords: Anti-DNA autoantibody; RNA aptamer

Various autoantibodies which are believed to be major pathogenic mediators in the development of autoimmune disease patients. In systemic lupus erythematosus (SLE) patients, high levels of anti-DNA autoantibodies seem to mediate many clinical manifestations of lupus (reviewed in [1–2]). Anti-DNA autoantibodies are tightly bound either to the double strand (ds) DNA/RNA and lead to dysfunctioning of various organs, especially serious kidney damage by glomerular deposition of immune complexes [3–7]. It is generally known that the pathogenic anti-DNA autoantibodies in SLE are of IgG class and preferentially bind to native dsDNA over to ssDNA [8]. Since anti-DNA autoantibodies are likely to recognize either the specific sequences or the conformations of nucleic acids, specific secondary structures generated by RNA molecules, if any, might also be recognized by the anti-DNA autoantibody. However, exact mechanism of immunogenicity of nucleic acids is not clearly elucidated [9–10].

Selection of the high affinity sequences among random pool of the nucleic acid library might provide a valuable clue for determining the fine specificity of the autoantigenic nucleic acid epitopes. Single-strand RNA conforms of stable and diverse 3-D structures by intra-molecular base-pairing, therefore, reiterated selection/evolution procedure could select the winner sequences or structures among random sequence RNA library. Selected nucleic acids using such procedure are generally named as ‘aptamers’ [11–12]. Because of huge diversity of the RNA library (1014–1015 molecules) and the ease of generating RNA molecules by in vitro enzyme reactions, RNA library has advantages over any biological or synthetic libraries in selecting high affinity aptamers [13]. Identification of these defined DNA/RNA epitopes could also provide a hint for the immunological cross-reactivity of self-antigens. Even though similar
approaches have been previously applied to determine the ligand specificity of the U1 snRNA binding autoantibody [14], there is no report on the in vitro selected aptamer for the anti-DNA autoantibody to our knowledge.

High affinity binding molecules for the autoantibody could also be developed as a valuable decoy inhibiting the pathogenic effect of anti-DNA autoantibodies. Decoy molecules would inhibit the binding of the autoantibody to the self-antigen and prevent the immune responses from being induced by the immune complex, reducing the development of autoimmunity. In fact, the specific peptides selected for the anti-DNA autoantibody were found to inhibit the autoantibody-mediated glomerular deposition in vivo [15,16]. Since one of the critical steps of the autoimmune response could be the proliferation of the autoantigenic B-cell clones, it is also expected that the decoy molecules could reduce the production of autoantibody by inhibiting proliferation of the autoantibody-secreting B-cell clone.

MRL/lpr is one of the well-established autoimmune-prone mouse strains, producing high levels of anti-DNA autoantibodies [17]. Since the molecular nature of anti-DNA autoantibodies from MRL/lpr mouse is extensively studied, it would be appropriate to use those autoantibodies as selection targets and biochemical analyses [18–19]. One such monoclonal autoantibody is the G6-9, which showed strong binding affinity to ssDNA and dsDNA [20]. In this paper, G6-9 anti-DNA autoantibody was used as the target for in vitro selection to study the fine specificity of the antigenic nucleic acids and to modulate the production and binding activity of the autoantibody.

Materials and methods

**Generation and purification of the anti-DNA autoantibody.** The splenocytes from a 24-week-old MRL/lpr mouse were fused with SP210 myeloma cell lines as described elsewhere [21]. Hybridoma clones that bound to dsDNA and ssDNA were selected using an ELISA method. One of the selected clones, G6-9 monoclonal cell line, was cultured in 300 ml for 7 days to obtain a milligram of antibodies. Monoclonal antibody was purified by an affinity chromatography using ssDNA-conjugated agarose beads (Gibco-BRL). Culture supernatant from the G6-9 cell line was loaded to the ssDNA agarose column and washed with 10 column volumes of PBS. The antibody was then eluted with 700 mM binding buffer with 30 μCi [α-32P]UTP (800 Ci/mmol), 50 μl T7 RNA polymerase, and 20 μl RNase inhibitor in 20 μl at 37°C. Reaction was terminated by 4 unit DNase RQ1 at 37°C for 15 min and electrophoresed on 6% polyacrylamide/7 M urea gel. RNA band was cut from the gel, incubated in 500 μl elution buffer (0.5 M CH3COONaH, 1 mM EDTA, and 0.2% SDS) for 3 h at 37°C, and purified by phenol/chloroform and ethanol precipitation. RNA was denatured for 5 min at 65°C and renatured for 20 min at room temperature. After measuring the incorporated radioactivity, the same count of radiolabeled RNA was mixed with mouse normal IgG or the G6-9 anti-DNA autoantibody in the binding buffer for 30 min at room temperature. Protein G-Sepharose beads were added to each solution and incubated for 30 min at room temperature with shaking. Resulting suspension was centrifuged and the supernatant was removed. Beads were washed five times with the binding buffer and bound RNA was eluted with 10 mM EDTA for 5 min at room temperature. Eluted RNA was purified by phenol/chloroform extraction followed by ethanol precipitation and subjected to electrophoresis on 6% polyacrylamide/7 M urea gel.

**Competitive ELISA assay.** Calf thymus dsDNA or the selected RNA aptamer was immobilized to the microtiter plates for overnight at 4°C and excess DNA or RNA was removed in vacuo. Hundred microliters of 3% BSA–PBST was added to the plates and allowed to stand for 2 h at room temperature. Anti-DNA autoantibodies (5 μg/ml) were pre-incubated with various competitors, such as random RNA pool (SE0), selected pool of RNA (SE22), cloned RNA aptamer (#9 RNA), and calf thymus dsDNA, and yeast tRNA. Pre-incubated mixture was transferred to the calf thymus dsDNA or the RNA aptamer coated microtiter plates and incubated for 2 h at room temperature. After washing plates three times with PBST, the alkaline phosphatase-conjugated anti-mouse IgG was added to the plate and incubated for 2 h at room temperature. After washing plates five times with the same buffer, 2 mM p-nitrophenyl phosphate solution (pH 10) was added and incubated for 2 h at room temperature. Absorbance was measured at 405 nm using an ELISA reader (Bio-Rad).

**Deletion analysis of RNA aptamer #9.** DNA template for the RNA aptamer #9 was digested with SacI and BamHI to remove T7 promoter to prevent the transcription of the full size RNA. Truncated DNA templates were generated by PCR with 100 ng DNA fragment, 1.5 mM MgCl2, 1 unit Taq polymerase, and 0.25 μM primer set. Primers for each PCR are the following: loop 0, 5’ primer (CCGTAAT ACGACCTACTATAAGGGAAGCTGGTACCAGATCT) 3’ primer (GGAGAATTCGAGCTCCCGATC); loop 1, 5’ primer (CCGTAAT ACGACCTACTATAAGGGAAGCTGGTACCAGATCT) 3’ primer.
(AAGAGTCACGGGGCAATGCTC); and loop 2, 5′ primer (CCGTAAACGACTCATTATAGCTGTCGGATGTCTTAGG); PCR fragments were purified and used as templates for the in vitro transcription with the radiolabeled [α-32P]UTP as described above. Radiolabeled RNA molecules were subjected to the gel electrophoresis, eluted from the gel, and used for the binding assay with anti-DNA autoantibody as described above.

**RNA footprinting assay.** In order to map the binding sites of the anti-DNA autoantibody within the selected aptamers, #9 RNA footprinting assay was performed. In vitro transcribed RNA was dephosphorylated by calf intestine phosphatase for 1 h at 37°C, extracted with phenol, and precipitated with ethanol. RNA was labeled with [α-32P]UTP and T4 polynucleotide kinase, electrophoresed on 6% polyacrylamide/7M urea gel, and eluted from the gel. Part of the labeled RNA was digested either with RNase T1 or with RNase S1. Alkaline hydrolysis of RNA was also performed in 50 mM NaHCO3 (pH 9.0), 1 mM EDTA, and 0.25 mg/ml tRNA for 15 min at 90°C to generate the control lane. Labeled RNA (62 nM) was denatured and renatured in the binding buffer [30 mM Tris–HCl (pH 7.5), 150 mM NaCl, 10 mM MgCl2, 2 mM DTT, and 1% BSA]. Anti-DNA autoantibody (RNA:protein molar ratio of 1:0, 1:0.5, 1:1, and 1:2) was added to the renatured RNA and incubated at 37°C for 10 min, and RNase T1 or RNase S1 was then added and incubated for another 10 min. After ethanol precipitation, dried pellet was resuspended in loading dye, denatured for 5 min at 65°C, and immediately cooled down on ice. Samples were loaded on 15% polyacrylamide/7M urea gel in 1× TBE solution for 4 h at 1500 V. The gel was dried and exposed on X-ray film for autoradiography.

**Results**

**Enrichment of the RNA aptamers to the G6-9 monoclonal anti-DNA autoantibody**

Monoclonal anti-DNA autoantibody G6-9 was produced from the MRL/lpr mouse, human SLE model mouse. G6-9 autoantibody was previously shown to strongly bind to the dsDNA [20]. In vitro selection and amplification procedures were repeated to acquire RNA aptamers with higher binding affinity than that of cognate antigen. Binding of radiolabeled RNA was assessed by immunoprecipitation either with normal mouse IgG (N) or with the G6-9 anti-DNA autoantibody (A), as shown in Fig. 1A. Bound RNA was extracted from the immunoprecipitates and loaded onto the gel to visualize the labeled RNA. To roughly estimate the binding affinity of RNA, 10% (lane 1) or 20% (lane 6) of input RNA was loaded onto the same gel. Selected RNA pool from the cycle 22 (SE22) binds strongly to the anti-DNA autoantibody (lane 5), but not to the normal IgG even with 10 times more amount of the normal antibody (lane 4). Since extremely stringent condition was applied after 10 cycles, high affinity RNA aptamer seems to be enriched in the later cycle of selection.

After 22 cycles of selection (SE22), SE22 RNA was converted to DNA and cloned into each aptamer. Sequence analysis of seventeen aptamer clones reveals two groups of RNA: 14 clones in group I and three clones in group II. Ten clones have exactly the same sequences.
and the other clones in the group have similar sequences with a few point mutations. Repeatedly selected sequence (aptamer #9) was chosen (Fig. 1A) for further analysis. High affinity RNA aptamers seem to be generated by the repeated in vitro selection procedure, which might bind to the variable region of the G6-9 anti-DNA without background binding to the constant region of the autoantibody.

Measurement of the binding affinity by the surface plasmon resonance

To quantify the binding affinity of the selected RNA to anti-DNA autoantibody in more detail, the surface plasmon resonance (SPR) technique was employed. SPR profiles to the G6-9 autoantibody are produced by subtracting background binding to control normal antibody (100 nM RNA/DNA, Fig. 1B). Selected RNA aptamer (#9) showed the highest binding and the SE22 RNA pool (SE22) also showed relatively high binding in comparison to random RNA pool (SE0). Binding of calf thymus dsDNA (dsDNA) and yeast tRNA (tRNA) was shown to be much lower than that of selected RNA, even though dsDNA was previously shown to be an antigen for the G6-9 autoantibody [20]. The SPR study clearly demonstrates that the selected RNA has a much higher binding affinity to the anti-DNA autoantibody than its cognate antigen.

Binding parameters of RNA and DNA were calculated by using BIA evaluation program (Table 1). To determine the accurate binding constants, at least six different concentrations of the RNA were used for each measurement. As expected, the binding affinity of the selected pool (SE22) was in nanomolar range. Aptamer #9 RNA showed the highest binding affinity ($K_D = 2 \times 10^{-9}$ M), affording at least 100-fold increase of affinity over that of the dsDNA ($K_D = 4 \times 10^{-7}$ M). The other RNA clones also demonstrated similar binding affinity to that of the selected pool. Binding parameters shown in Table 1 are in good agreement with the equilibrium binding assay with radiolabeled RNA and various concentrations of the autoantibody as in Fig. 1B.

### Table 1

<table>
<thead>
<tr>
<th>Original RNA pool</th>
<th>$k_s$ (M$^{-1}$ s$^{-1}$)</th>
<th>$k_d$ (s$^{-1}$)</th>
<th>$K_D$ (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SE22 RNA pool</td>
<td>2.0 $\times$ 10$^4$</td>
<td>6.0 $\times$ 10$^{-3}$</td>
<td>3.0 $\times$ 10$^{-2}$ (±0.5)</td>
</tr>
<tr>
<td>Aptamer #9 (Group I)</td>
<td>4.1 $\times$ 10$^5$</td>
<td>1.9 $\times$ 10$^{-3}$</td>
<td>5.0 $\times$ 10$^{-2}$ (±2.3)</td>
</tr>
<tr>
<td>Aptamer #22 (Group II)</td>
<td>3.5 $\times$ 10$^5$</td>
<td>7.1 $\times$ 10$^{-4}$</td>
<td>2.0 $\times$ 10$^{-3}$ (±0.4)</td>
</tr>
<tr>
<td>Aptamer #8 (Group I)</td>
<td>2.4 $\times$ 10$^5$</td>
<td>6.4 $\times$ 10$^{-3}$</td>
<td>2.7 $\times$ 10$^{-3}$ (±1.5)</td>
</tr>
<tr>
<td>Aptamer #16 (Group II)</td>
<td>1.2 $\times$ 10$^6$</td>
<td>6.2 $\times$ 10$^{-3}$</td>
<td>5.4 $\times$ 10$^{-9}$</td>
</tr>
<tr>
<td>Calf thymus dsDNA</td>
<td>2.5 $\times$ 10$^5$</td>
<td>1.0 $\times$ 10$^{-3}$</td>
<td>4.0 $\times$ 10$^{-3}$</td>
</tr>
<tr>
<td>Sonicated dsDNA</td>
<td>2.2 $\times$ 10$^5$</td>
<td>1.0 $\times$ 10$^{-3}$</td>
<td>4.5 $\times$ 10$^{-7}$</td>
</tr>
<tr>
<td>Yeast tRNA</td>
<td>6.0 $\times$ 10$^5$</td>
<td>6.3 $\times$ 10$^{-3}$</td>
<td>1.1 $\times$ 10$^{-6}$</td>
</tr>
<tr>
<td>Yeast tRNA</td>
<td>6.7 $\times$ 10$^5$</td>
<td>1.3 $\times$ 10$^{-3}$</td>
<td>2.0 $\times$ 10$^{-4}$</td>
</tr>
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Evaluation of $k_s$, $k_d$, and $K_D$ values was performed by the BIA analysis program; the binding parameters which satisfy the $\chi$ and $T$ values are displayed in the table.

Determination of structural binding motif of the aptamer #9 RNA

To understand the structural nature of RNA aptamer and autoantibody interaction, secondary structures of selected RNA clones were predicted by mfold program. Application of the energy minimization program to the aptamer #9 RNA predicted that most of the selected sequences form the structure with two stem-loops (SL1 and SL2) along with a small bulge (B) between them (Fig. 2A).

To determine how much structural elements of RNA are required for the proper binding, parts of #9 RNA were deleted to generate smaller versions of folded RNA (Fig. 2A). Truncation of RNA does not seem to change the domains of the RNA, because deleted RNA molecules were predicted to fold into stable sub-structures of #9 RNA (data not shown). Five different deletion clones were prepared: SL0 clone which spans 5’ constant region, SL1, SL2, SL1/2, and SL1/2/S* clones which span the random nucleotide region (Fig. 2A). Relative binding affinities of these truncated RNA were measured by the immunoprecipitation assay (Fig. 2B). It demonstrates that SL1/SL2 RNA which spans most of the random region bound to the autoantibody (lanes 7–10), but neither SL1 nor SL2 alone did (lanes 3–6). It could be due to the fact that the autoantibody recognizes the whole structure made up of two stem-and-loops (SL1 and SL2) and the internal bulge (B), but not individual sub-structures. It is also interesting to note that the SL0, generated entirely by the 5’ defined sequences of library, did not bind to the autoantibody at all (lanes 1–2). It is likely that the common sequences at the ends of the RNA library do not contribute to the autoantibody binding; only the specific sequences corresponding to the random region of the library drive the effective binding to the autoantibody during the selection procedure.

To further characterize the binding pattern of autoantibody to the selected RNA, RNase footprinting analysis was performed with aptamer #9 RNA. RNA was end labeled, bound to various concentrations of autoantibody, and subjected to RNase digestions
Fig. 2. Determination of the minimal binding domain. (A) Domain structure of the aptamer #9 RNA, having a stable conformation as predicted by mfold program. Random sequences spans the nucleotides 21–91) conform of two stem-and-loops (SL1 and SL2), small bulge (B), and the flanking stem (S*). Defined sequences at the 5' of the random sequence form another stem-and-loop, as denoted by SL0. Nucleotides at the junction of the predicted domains were the following: SL0, 1–24; SL1, 42–61; SL2, 65–91; SL1/2, 28–91; and SL1/2/ S*, 28–102. (B) Domain mapping of the aptamer #9 RNA. Deleted RNA molecules (SL0, SL1, SL2, SL1/2, and SL1/2/S*) as well as the full-length aptamer #9 RNA (#9) were prepared as described in Materials and methods. Each RNA was radiolabeled and immunoprecipitation with the G6-9 autoantibody and loaded onto the even number lanes of the gel (lanes 2, 4, 6, 8, 10, and 12). In odd number lanes (lanes 1, 3, 5, 7, 9, and 11), one-tenth of the labeled RNA was loaded as an input control for each deleted RNA molecule.

Fig. 3A). Diagram of aptamer #9 RNA with RNase sensitive sites is shown in Fig. 3B. It clearly shows two stem-and-loop structures (SL1 and SL2), along with long double-strand stem structure (S* and SL0). Addition of the G6-9 autoantibody induces a drastic conformational change to SL0 and S* (nucleotides 25–28), accompanied by RNase protection in SL1 and SL2 (nucleotides 40–91) (lanes 4–6 and lanes 8–10), suggesting a binding of antibody in SL1/SL2 with concomitant alteration of the RNA structure. RNase footprinting analysis is in agreement with that from the deletion mapping shown in Fig. 2, suggesting that the SL1/SL2 region which was formed by most of the selected sequences seems to be a binding motif for the G6-9 autoantibody. More detailed analysis of the aptamer might provide information on the structure of autoantibody binding nucleic acids.
Specific binding of the RNA aptamer #9 to various anti-DNA autoantibodies

Since the aptamer was selected against the G6-9 monoclonal antibody as a specific target, it is interesting to test whether it could bind to the G6-9 with the highest affinity among various monoclonal antibodies. To directly assess the specificity of the selected RNA, radiolabeled RNA aptamer #9 was tested for its binding to three different anti-DNA autoantibodies. They were extensively characterized for their antigen specificities; all of them were found to have high dsDNA binding affinities but distinct ssDNA and RNA binding patterns [18,20]. G6-9 and G5-17 were found to have high to moderate ssDNA binding affinity but no RNA binding, whereas G1-21 showed low ssDNA binding and high RNA binding affinities. Data shown in Fig. 4A clearly demonstrate that the binding affinities of the RNA aptamer on three autoantibodies are quite different. It strongly binds to its selection target G6-9 as expected, but it shows weak binding to G5-17 and no binding to G1-21. One of the monoclonal catalytic antibodies (4f4f) was also tested for the binding, because some of them were known to have similar characteristics of autoantibodies [23]. It is interesting to note that RNA aptamer #9 selectively distinguishes G6-9 and G5-17 whose binding patterns were known to be similar. Therefore, the selected RNA seems to have a specific binding structure which could not be recognized by other similar autoantibodies. Such a high specificity of the selected RNA aptamer might be useful to distinguish similar autoantibodies in serum.

Inhibition of DNA binding to the anti-DNA autoantibody by the aptamer RNA

Since the RNA aptamer specifically binds to the G6-9 autoantibody, it is expected to inhibit its cognate antigen binding to the autoantibody and prevent the anti-

Fig. 4. Specific inhibition of the anti-DNA autoantibody binding to the DNA by the selected RNA aptamer. (A) Specific binding of the aptamer #9 to the G6-9 autoantibody. RNA was radiolabeled and immunoprecipitated with various lines of monoclonal anti-DNA autoantibodies (G6-9, G1-21, and G5-17, lanes 3–5) and a catalytic antibody 4f4f (lane 6). Background binding to the normal IgG (lane 2) was also assessed. (B) Inhibition of DNA binding by the aptamer. Radiolabeled SE22 RNA was immunoprecipitated either with the normal IgG (N, lane 2) or with the G6-9 autoantibody (A, lanes 3–9). Increasing amount of the non-labeled SE22 RNA (lanes 4–6) or the calf thymus DNA (lanes 7–9) was included as competitor in each immunoprecipitation reaction. One-tenth of labeled RNA was loaded in the lane 1 as an input control.

Fig. 5. Competition of the G6-9 autoantibody binding to DNA by the selected RNA pool and the aptamer #9. (A) Competitive ELISA with the immobilized aptamer #9. Competitive ELISA was performed by pre-incubating the G6-9 autoantibody with various concentrations of competitors, followed by incubation with the immobilized aptamer #9 RNA. Relative binding was presented as a % binding, in comparison to the 100% binding without any competitor. Representative data from three independent experiments are presented. (B) Competitive ELISA with the immobilized calf thymus DNA. G6-9 autoantibody was pre-incubated with various concentrations of competitor RNA (tRNA, SE0 RNA, SE22 RNA, and aptamer #9 RNA). Antibody–RNA complex was incubated with the plates with immobilized calf thymus dsDNA and the amount of bound antibody was measured by the absorbance at 405 nm. Symbols for competitors are following: square, tRNA; circle, dsDNA; triangle, SE0; diamond, and SE22; reverse triangle, aptamer #9.
RNA aptamer, binding of the dsDNA to the autoanti-
body complex was efficiently inhibited by competitive inhibitors (lanes 3–6). However, challenging the RNA-autoanti-
temine interaction motifs on the surface of the autoantibody cognate antigen dsDNA does not work competi-
tively. Even with the cognate dsDNA, the non-cognate RNA aptamer #9 was immobilized to the plate, while G6-9 anti-DNA autoantibody was incubated in solution with various RNAs and DNAs as competitors. Binding of aptamer #9 RNA to the autoantibody was efficiently competed either by selected RNA pool (SE22) or by aptamer #9, as expected. However, when the calf thymus dsDNA or random RNA pool (SE0) was used as competitors, the binding of the anti-DNA autoantibody was not competitively inhibited. It is more pronounced by yeast tRNA competitor, which shows no competition even in high concentration. It suggests that the binding of selected aptamer #9 is quite specific, thus its binding cannot be inhibited by random RNA pool (SE0), tRNA, even with the cognate antigen dsDNA. Similar result was obtained by immobilizing dsDNA and competing for the autoantibody binding with other RNAs (Fig. 5B). Aptamer #9 RNA and selected pool RNA (SE22) showed higher competition for the binding to the dsDNA-autoantibody complex, but neither tRNA nor calf thymus dsDNA did. Competitive ELISA showed that selected RNA pool and aptamer #9 RNA could efficiently inhibit the autoantibody binding to its cognate antigen in vitro.

Discussion

It is generally believed that RNA could fold into specific binding pockets for proteins, mimicking protein–protein interaction motifs on the surface of the protein [24–25]. In fact, RNA aptamer has to bind to the epitope binding site of the autoantibody to be used as a specific blocker. Selected RNA aptamer strongly binds to the G6-9 autoantibody that was used as a selection target, but not to other similar autoantibodies. Considering similar binding patterns of various monoclonal anti-DNA autoantibodies, it is amazing for the RNA aptamer to distinguish epitope binding sites of similar autoantibodies. In addition, when dsDNA antigen–autoantibody immune complex was challenged by the RNA aptamer, binding of the dsDNA to the autoanti-
body was specifically inhibited. These results clearly demonstrate that the RNA aptamer binds more specifically and strongly to anti-DNA autoantibody than cognate antigen dsDNA does, which makes it possible to utilize the selected RNA aptamer as a specific blocking agent for the anti-DNA autoantibody in the autoimmune system.

Even though the selection of the peptide sequence for the anti-DNA autoantibody was previously reported [15], our paper is the first report to use nucleic acid aptamer against the anti-DNA autoantibody to the best of our knowledge. Our result presented in this paper demonstrated that the RNA aptamer was even more effective than peptide aptamers in inhibiting autoanti-
temine interaction motifs on the surface of the autoantibody cognate antigen dsDNA does not work competi-
tively. Even with the cognate dsDNA, the non-cognate RNA aptamer #9 was immobilized to the plate, while G6-9 anti-DNA autoantibody was incubated in solution with various RNAs and DNAs as competitors. Binding of aptamer #9 RNA to the autoantibody was efficiently competed either by selected RNA pool (SE22) or by aptamer #9, as expected. However, when the calf thymus dsDNA or random RNA pool (SE0) was used as competitors, the binding of the anti-DNA autoantibody was not competitively inhibited. It is more pronounced by yeast tRNA competitor, which shows no competition even in high concentration. It suggests that the binding of selected aptamer #9 is quite specific, thus its binding cannot be inhibited by random RNA pool (SE0), tRNA, even with the cognate antigen dsDNA. Similar result was obtained by immobilizing dsDNA and competing for the autoantibody binding with other RNAs (Fig. 5B). Aptamer #9 RNA and selected pool RNA (SE22) showed higher competition for the binding to the dsDNA-autoantibody complex, but neither tRNA nor calf thymus dsDNA did. Competitive ELISA showed that selected RNA pool and aptamer #9 RNA could efficiently inhibit the autoantibody binding to its cognate antigen in vitro.

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