A Study on Cross-Reactivity of Anti-DNA Antibody with Glycosaminoglycans

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To study the pathogenesis of lupus nephritis, the cross reactivity between anti-DNA antibody and glycosaminoglycans (GAGs) was investigated. Monoclonal anti-DNA antibodies were obtained from hybridomas by the fusion of MRL-lpr/lpr splenocytes with murine myeloma cells. Some of these monoclonal anti-DNA antibodies showed cross reactivity with GAGs, such as hyaluronic acid, chondroitin sulfate and heparan sulfate. To elucidate the mechanism of cross reactivity, inhibition assays with propanol and polyethylenimine (PEI), a cationic agent, were carried out. Increase of the concentration of PEI (0.6-2.0 % vol/vol) resulted in a dose dependent decrease in the binding ability of anti-DNA antibody to GAGs. Propanol, an organic reagent which disrupts the van der Waals bonds between epitopes and paratopes, showed little inhibitory effect on the binding activity of monoclonal anti-DNA antibody to GAGs. These results indicate that the binding of anti-DNA antibody to GAGs is due to a charge interaction rather than van der Waals forces. Anti-DNA antibody which can react with GAGs in the glomerular basement membrane seems to play an important role in the pathogenesis of lupus nephritis.

Key words: anti-DNA antibody, cross-reactivity, glycosaminoglycan, lupus nephritis

High titer of anti-DNA antibody, especially anti-double-stranded DNA antibody activity in the sera is specific for the patients of systemic lupus erythematosus (SLE) and correlates with clinical findings and severity of renal involvement (1). However, the pathogenic significance of anti-DNA antibody in lupus nephritis remains uncertain.

There are two proposed mechanisms by which anti-DNA antibody could cause lupus nephritis. One is the circulating immune complex (CIC) mechanism: circulating DNA/anti-DNA antibody immune complex is present in the sera of patients with lupus nephritis. However, the direct demonstration of DNA both in CIC and glomeruli has been difficult, and reported only in a few studies (2, 3). Another mechanism is the in situ IC formation mechanism: Izui demonstrated that DNA had a high affinity for collagen molecule in GBM in vitro (4). They proposed that circulating anti-DNA antibodies would bind to DNA bound to GBM and form the immune complex in situ. However, there is a lack of direct and concrete evidence which supports either of these two hypotheses.

Recently, Faaber et al. showed the cross reactivity of anti-DNA antibody with GAGs (5, 6), and proposed a new hypothesis with respect to the pathogenesis of lupus nephritis. They suggest that anti-DNA antibody binds directly to the GAGs to form the immune complex in situ and causes lupus nephritis.

We cultivated the monoclonal anti-DNA antibodies and studied the cross reactivity with GAGs to investigate the precise mechanism of this cross reactivity.

Materials and Methods

Mice: MRL-lpr/lpr mice were purchased from Kiwa laboratory, Japan and BALB/C mice were obtained from the animal colony of our institute.

Cell fusion and cloning. Somatic cell hybridization was carried...
out as described by Köhler and Milstein (7). Spleen cells from MRL-Lpr lpr mice were fused with the non-immunoglobulin-secreting myeloma cell line, NS1. Anti-DNA antibody-producing hybridomas were cloned by repeating the procedure of limiting dilutions.

**Results**

Production of monoclonal anti-DNA antibodies. The cell-fusion procedure was carried out twice and yielded 98 cell lines producing anti-DNA antibody.

Cross-reactivity of monoclonal anti-DNA antibodies with GAGs. Seven clones of anti-DNA antibody producing hybridomas were able to cross-react with GAGs (Table 1). Anti-DNA antibody activity of these clones was also confirmed with Crithidia assay. There was a correlation between anti-DNA activity and anti-GAG activity in these monoclonal antibodies (Figs. 1, 2). This data indicate that bindings of these monoclonal antibodies to DNA and GAGs were not mediated with non-specific charge interaction because binding activities were not significantly reduced with 0.3 M NaCl in which concentration, non-specific charge interaction was inhibited (8). It was also demonstrated that NaCl at the concentrations used in this study did not dissociate the DNA or GAG from the plate (Data was not shown).

**Table 1**

<table>
<thead>
<tr>
<th>Hybridoma clones</th>
<th>Heparin sulfate</th>
<th>Chondroitin sulfate</th>
<th>Hyaluronan</th>
<th>DNA</th>
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<tr>
<td>C51</td>
<td>0.209</td>
<td>0.433</td>
<td>0.699</td>
<td>0.839</td>
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<td>LAH</td>
<td>1.273</td>
<td>1.820</td>
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<td>1.607</td>
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<td>4C2</td>
<td>0.350</td>
<td>0.508</td>
<td>0.315</td>
<td>0.667</td>
</tr>
<tr>
<td>581</td>
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<td>1.144</td>
<td>1.096</td>
<td>1.015</td>
</tr>
<tr>
<td>388</td>
<td>1.779</td>
<td>1.882</td>
<td>1.517</td>
<td>1.746</td>
</tr>
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<td>0.605</td>
<td>0.894</td>
<td>1.089</td>
</tr>
<tr>
<td>7C1</td>
<td>0.759</td>
<td>1.056</td>
<td>0.326</td>
<td>1.345</td>
</tr>
</tbody>
</table>

The presence of increasing amount of PEI caused dose-dependent reduction in anti-DNA and anti-GAG activity (Fig. 4). Five % (vol/vol) of PEI reduced anti-DNA activity and anti-GAG activity by 50 % and 65 %, respectively. But PEI did not show an inhibitory effect on the other antigen-antibody interaction: microsomal antigen and monoclonal anti-microsomal antibody.

**Effect of PEI on the binding to DNA and GAG.**

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**Fig. 1** Correlation between anti-DNA activity and anti-heparin sulfate activity of monoclonal anti-DNA antibodies.

**Fig. 2** Correlation between anti-DNA activity and anti-chondroitin sulfate activity of monoclonal anti-DNA antibodies.

**Fig. 3** Influence of NaCl concentration on the binding of monoclonal anti-DNA antibodies to DNA and GAG. Decrease of binding activity to DNA and GAG in the presence of increasing concentration of NaCl in a dose-dependent manner was demonstrated (Fig. 3). These data indicate that bindings of these monoclonal antibodies to DNA and GAGs were not mediated with non-specific charge interaction because binding activities were not significantly reduced with 0.3 M NaCl in which concentration, non-specific charge interaction was inhibited (8). It was also demonstrated that NaCl at the concentrations used in this study did not dissociate the DNA or GAG from the plate (Data was not shown).

**Fig. 4** Influence of polyethyleneimine on the binding of monoclonal anti-DNA antibodies to DNA, chondroitin sulfate and heparin. Binding of monoclonal anti-DNA antibody (1.0% clone to DNA) (c) and heparin sulfate (g). Binding of monoclonal anti-DNA antibody to DNA was reduced (a) and heparin sulfate was inhibited (e). Binding of monoclonal anti-DNA antibody to heparin sulfate was inhibited (e).
phospholipids. Phosphodiester-linked phosphate groups were identified as possible cross-reactive moieties (13-15). Furthermore, cross-reactivity of anti-DNA antibody extended to a variety of antigens, such as IgG, platelet, Raji cell and endogenous bacteria (16-18). Faifer et al. also reported the cross-reactions with GAGs (5, 6). The wide variety of cross-reactions is difficult to explain with the existing data. This situation seems rich in possibilities for further investigations.

The repulsive and attractive forces that constitute the non-covalent interaction between antibodies and antigens (10) are characterized by a) Dispersion (van der Waals) force and b) Electrostatic (Coulombic) interaction. The organic reagents, propanol and acetic acid, decrease the surface tension of the liquid medium and disrupt the van der Waals bond to dissociate the antigen-antibody complex. Microsomal antigen and anti-microsomal antibody complex is efficiently dissociated by propanol. However, the binding of monoclonal anti-DNA antibodies to DNA and GAG were not inhibited by propanol at the concentration in which microsomal antigen and anti-microsomal antibody was dissociated. From these data, it may be concluded that van der Waals force does not contribute the interaction between anti-DNA antibody and DNA or GAG.

Both DNA and GAG are negatively charged. Edling and Hahn reported that anti-DNA antibodies eluted from NZB/W F1 mouse kidneys had higher isoelectric points than those in the sera. They speculated that cationic anti-DNA antibodies were pathogenic in lupus nephritis (19). In the present study, the cationic agent, PEL, inhibited the binding of anti-DNA antibody to DNA and GAG. It is possible that the binding of anti-DNA antibody to GAG is mediated by simple charge-charge interaction. Because the 0.3M ionic strength, which can prevent non-specific charge-charge interaction did not affect the interaction between anti-DNA antibody and GAG, this interaction does not seem to be mediated with a simple force between opposite charges. From these results, it appears that this charge-charge interaction exists between epitope and paratope.

Van der Waals force decreases at a rate inverse to the 7th power of the intermolecular distance. The distance between two molecules should be very close for van der Waals force to work efficiently. On the other hand, electrostatic force is inversely proportional to the second power of the intermolecular distance and could work at distances where van der Waals force could not work.

There was a significant correlation between anti-DNA activity and anti-GAG activity in these monoclonal antibodies. However, DNA and GAG do not have similar molecular structures other than repeating negative-charged residues. It seems to be reasonable that van der Waals force does not work effectively between such molecules.

In this study, the cross-reactivity of monoclonal anti-DNA antibody with GAG was demonstrated under various conditions. Heparan sulfate proteoglycan, which is one of the GAGs in glomeruli, is an important component of anatomic site in the kidney and necessary to maintain both charge and size selective barriers. (20, 21). The cross-reaction between anti-DNA antibody with GAG may play two possible roles in the pathogenesis of lupus nephritis. Direct binding of anti-DNA antibody to GAG may lead to the formation of the immune complex in situ and cause the activation of components and tissue damage. Another possibility is that anti-DNA antibody may neutralize the negative charge of GAG to disturb the charge-selective barrier and result in proteinuria.

References


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