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Anti-DNA antibody mediated catalysis is isotype dependent

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ABSTRACT

Anti-DNA antibodies are the serological hallmark of systemic lupus erythematosus, and participate in the pathogenesis of lupus nephritis by cross-reacting with multiple renal antigens. Previously, using a panel of murine anti-DNA IgGs that share identical variable regions but that differ in the constant regions, we demonstrated that the cross-reaction and renal pathogenicity of anti-DNA antibodies are isotype dependent. In this study, we investigated the catalytic potential of this anti-DNA antibody panel, and determined its isotype dependency. The three isotype switch variants (IgG1, IgG2a, IgG2b) and the parent IgG3 PL9-11 anti-DNA antibodies were compared in their catalysis of 500 base pair linear double stranded DNA and a 12-mer peptide (ALWPPNLHAWVP), by gel analysis, MALDI-TOF mass spectrometry, and nuclear magnetic resonance spectroscopy. The binding affinity of anti-DNA antibodies to double stranded DNA and peptide antigens were assessed by ELISA and surface plasmon resonance. We found that the PL9-11 antibody isotypes vary significantly in their potential to catalyze the cleavage of both linear and double stranded DNA and the proteolysis of peptides. The degree of the cleavage and proteolysis increases with the incubation temperature and time. While different PL9-11 isotypes have the same initial attack sites within the ALWPPNLHAWVP peptide, there was no correlation between binding affinity to the peptide and proteolysis rates. In conclusion, the catalytic properties of anti-DNA antibodies are isotype dependent. This finding provides further evidence that antibodies that share the same variable region, but which have different constant regions, are functionally distinct. The catalytic effects modulated by antibody constant regions need to be considered in the design of therapeutic antibodies (abzymes) and peptides designed to block pathogenic autoantibodies.

1. Introduction

Systemic lupus erythematosus (SLE) is a potentially severe autoimmune disease characterized by increased titers of serum autoantibodies. Among these pathogenic autoantibodies, anti-DNA IgGs attract particular attention for their pivotal role in the pathogenesis of lupus nephritis, one of the most serious complications of SLE (Yung and Chan, 2008). The nephritogenicity of anti-DNA antibodies may be caused by their indirect binding to kidney tissue mediated by DNA/nucleosomes, or by direct binding to glomerular antigens mediated by antigenic cross-reactivity (Hanrotel-Saliou et al., 2011). The interactions between anti-DNA antibodies and glomerular resident cells can activate the complement cascade, modulate gene expression, enhance cellular proliferation, and alter cellular phenotypes (Jang et al., 2009; Qing et al., 2008; Yung et al., 2005, 2009; Zhang et al., 2012). Therefore, elucidation of the mechanisms through which anti-DNA antibodies interact with self-antigens is crucial in the development of novel therapeutics for lupus nephritis and other disease manifestations.

Previous studies suggested that the pathogenicity of anti-DNA antibodies in SLE patients and lupus animal models is isotype dependent (Bijl et al., 2002; Baudino et al., 2006; Krishnan et al., 2012). Recently, by using class switching in vitro, we generated a panel of monoclonal antibodies (mAb) from the murine PL9-11 IgG3 anti-DNA antibody. Members of the PL9-11 mAb panel

Abbreviations: AHS, AHSANNFNVKGI; ALW, ALWPPNLHAWVP; ds, double stranded; ELISA, enzyme-linked immunosorbent assay; mAb, monoclonal antibody; NMR, nuclear magnetic resonance; SLE, systemic lupus erythematosus; SPR, surface plasmon resonance.

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share identical variable regions, but differ from each other in the heavy chain constant region. Immunologic dogma has been that the variable regions in heavy chains and light chains are the sole structures that determine the binding of antibodies to antigens. However, our results showed that both antigenic specificity and renal pathogenicity differ between these PL9-11 derived mAbs (Xia et al., 2012), and that such differences arise from the different constant regions that may alter antibody secondary structure and function (Xia et al., 2013). Thus, anti-DNA antibodies can exhibit isotype dependent properties in binding to DNA or in cross-reaction with non-DNA antigens.

Catalysis of nucleic acids or peptide cleavage is an intrinsic property of certain antibodies associated with antigen binding. Increasing evidence suggests important functional roles for catalytic antibodies in homeostasis, autoimmune disease, and protection against infection (Paul et al., 2012; Tomin et al., 2015; Barrera et al., 2009; Nevinsky and Buneva, 2010). Interestingly, in autoimmunity, natural antibody-enzymes (abzymes) can have both beneficial and detrimental effects, depending on the specific disease and the targets they cleave (for comprehensive review, see Belogurov et al., 2009).

Since DNA-catalyzing antibodies were first described by Shuster et al. (1992), there has been increasing interest in the importance of antibodies with this effect in the pathogenesis of SLE. Remarkably, the concentrations of double stranded (ds) and single stranded (ss) DNA-hydrolyzing autoantibodies are elevated in the sera of patients with SLE and in murine lupus models, suggesting a role in disease (Nevinsky and Buneva, 2002; Kostrikina et al., 2011; Ponomarenko et al., 2002). Catalyzing anti-DNA antibodies may share with DNase I several similar, or even identical, amino acid residues, which are necessary for DNA hydrolysis and the binding of magnesium and calcium ions (Kostrikina et al., 2014). Indeed, purified anti-DNA antibodies can enter the nuclei of tumor cells and kill cells through DNA hydrolyzing mechanisms (Kozyr et al., 2002). Moreover, the cytotoxic effect and the DNA hydrolyzing activity of anti-DNA antibodies is enriched in the antibody fractions that display cross-reactivity with nuclear matrix proteins (Kozyr et al., 2000), suggesting an important role of binding specificity in the catalytic potential of these antibodies. However, there has not been a systematic study of the relationship between the catalytic activity and binding affinity or specificity of anti-DNA antibodies. Since we found that heavy chain constant regions affect the binding of anti-DNA antibodies (Xia et al., 2012, 2013), the present study was designed to investigate the influence of such binding alterations on catalytic activity by taking advantage of the PL9-11 anti-DNA mAb panel that share identical variable regions.

2. Methods

2.1. Antibodies and antigens

The murine IgG1, IgG2b, and IgG2a isotype variants were generated from the parent hybridoma clone of PL9-11 (IgG3) by class switching in vitro, as described (Xia et al., 2012). All isotypes share the original PL9-11 heavy and light chain V regions, but differ in the identity of the heavy chain C region. As described previously, PL9-11 mAbs were purified from culture supernatant, and normalized to the same concentration using a goat anti-mouse mAb which bound to the identical kappa chain shared by all members of the panel (Xia et al., 2012). The murine IgG3, IgG1, IgG2b, and IgG2a isotype controls (Southern Biotech, Birmingham, AL) showed no in vitro binding to dsDNA (Xia et al., 2012). The Fab and $F(ab')_2$ fragments were prepared from the PL9-11 mAb by commercial kits (Thermo Scientific, Rockford, IL).

Double stranded (ds) DNA (500 bp) was obtained from plasmid DNA (pHC-msCu vector) by restriction enzyme digestion, as described previously (Xia et al., 2013). Single stranded (ss) DNA (18 bp, TATAGCGCGCGCTATAT) was synthesized by Invitrogen (Carlsbad, CA).

Two 12 amino acid peptides (ALWPPNLHAWVP, or "ALW"; AHSANNFNVKGI, or "AHS") used in this study were identified by screening a Ph.D.™-12 phage display library (New England Biolabs, Ipswich, MA) with the PL9-11 mAb panel, and selecting a shared sequence bound by all isotypes (Xia et al., 2015). The 12-mer APNQHTPPWMLK peptide, which is specific for the murine non-DNA binding 3E5 mAb, served as a negative control. Unlabeled peptides were synthesized by the Rockefeller University Proteomics Resource Center (New York, NY). For isotope labeling of ALW, BL21(DE3) competent *Escherichia coli* (Ipswich, MA) were transformed with the pET-31b vector (EMD Millipore, Billerica, MA) that expresses ketosteroid isomerase-ALW peptide-6 × histag fusion protein (GENEWIZ Inc., South Plainfield, NJ). *E. coli* cells were grown in M9 media supplemented with $^{15}NH_4Cl$ and [^{13}C] D-glucose. After induction with isopropyl-beta-D-thiogalactopyranoside, cells were harvested and then lysed in Tris–HCl buffer (6 M guanidine, pH 7.9). A HisPur Ni-NTA resin kit (Thermo Scientific) was used for the isolation of the fusion protein, followed by cyanogen bromide cleavage (Zerfaß et al., 2014). After the removal of precipitated ketosteroid isomerase, the ALW peptide was lyophilized. The purity and effectiveness of labeling (>90%) was confirmed by MALDI-TOF mass spectrometry (data not shown).

2.2. Enzyme-linked immunosorbent assay (ELISA)

The dsDNA ELISA was performed as previously described (Gao et al., 2009; Xia et al., 2012), using the prepared linear dsDNA to coat the plates. Alkaline phosphatase-conjugated IgG goat anti-mouse κ chain was the secondary antibody.

2.3. DNA gel analysis

dsDNA was pre-incubated with PL9-11 mAbs at differential molar ratios and temperatures. Samples were loaded on a 0.8% gel of high-resolution agarose (National Diagnostics, Charlotte, NC). The running conditions were 70 V for 120 min in a B1A Mini electrophoresis chamber (Owl Separation Systems, Portsmouth, NH). Ethidium bromide (0.5 μg/ml) was used as a fluorescent indicator.

2.4. Surface plasmon resonance (SPR)

Using a Biacore 3000 instrument (Biacore, Piscataway, NJ), SPR analysis was performed to determine the binding affinity of PL9-11 IgGs to the different antigens (Xia et al., 2012). In brief, the antibodies were immobilized on a CM sensor chip (GE Healthcare, Port Washington, NY) at a concentration of 10 nM in MES buffer. The ALW and AHS peptides and the 18 bp ssDNA fragment (0–250 nM) in HEPES buffer (pH 7.4, with 0.05% Tween 20) were injected over the chip. The simple Langmuir model ($A + B \leftrightarrow AB$) was used for the calculation of binding kinetics, including association (k_{on}) and dissociation (k_{off}) rates. The times associated with the SPR flow are very short (<5 min) compared to the rates of hydrolysis observed (>30 min), and so the binding is assumed to be to unhydrolysed materials.

2.5. Nuclear magnetic resonance (NMR)

NMR was performed as described previously (Janda et al., 2012). In brief, the isotope labeled ALW peptide was dissolved in NMR buffer (10 mM MES, 90 mM NaCl, pH 6.0). ALW (36–100 μM) and each of the IgGs (18–50 μM) were mixed at molar ratios of 2:1 just