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The constant region affects antigen binding of antibodies to DNA by altering secondary structure

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ABSTRACT

We previously demonstrated an important role of the constant region in the pathogenicity of anti-DNA antibodies. To determine the mechanisms by which the constant region affects autoantibody binding, a panel of isotype-switch variants (IgG1, IgG2a, IgG2b) was generated from the murine PL9-11 IgG3 autoantibody. The affinity of the PL9-11 antibody panel for histone was measured by surface plasmon resonance (SPR). Tryptophan fluorescence was used to determine wavelength shifts of the antibody panel upon binding to DNA and histone. Finally, circular dichroism spectroscopy was used to measure changes in secondary structure. SPR analysis revealed significant differences in histone binding affinity between members of the PL9-11 panel. The wavelength shifts of tryptophan fluorescence emission were found to be dependent on the antibody isotype, while circular dichroism analysis determined that changes in antibody secondary structure content differed between isotypes upon antigen binding. Thus, the antigen binding affinity is dependent on the particular constant region expressed. Moreover, the effects of antibody binding to antigen were also constant region dependent. Alteration of secondary structures influenced by constant regions may explain differences in fine specificity of anti-DNA antibodies between antibodies with similar variable regions, as well as cross-reactivity of anti-DNA antibodies with non-DNA antigens.

1. Introduction

Antibodies (Abs) to DNA play a key role in the pathogenesis of systemic lupus erythematosus (SLE), in particular lupus nephritis. Abs to DNA are believed to contribute to renal injury in SLE patients by cross reactivity with glomerular antigens (Yung and Chan, 2008). Other mechanisms of pathogenicity include renal damage by penetration into living cells with modulation of gene expression and cell metabolism (Jang et al., 2009; Qing et al., 2008), enhancement of kidney cell proliferation (Yung et al., 2005), and induction of phenotypic changes in mesangial cells (Yung et al., 2009; Zhang et al., 2012). However, not all Abs to DNA share the same pathogenic potential. Certain subclasses of anti-double stranded (ds)DNA Abs are more closely associated with pathogenic potential, including human IgG1/IgG3 (Ravirajan et al., 2001), and mouse IgG2a in a murine lupus model (Savitsky et al., 2010). IgG subclass distribution of Abs to DNA differs between renal and extra-renal flares in patients with SLE (Bijl et al., 2002), while IgG3 deficient MRL/lpr mice have attenuated glomerulonephritis and prolonged survival (Greenspan et al., 2012). In addition, mice injected with monoclonal Abs (mAbs) with similar specificity for DNA but different IgG subclass and different relative affinity for basement membrane, showed unique patterns of IgG deposition in glomeruli and different levels of proteinuria (Krishnan et al., 2012). Therefore, the isotypes of Abs to DNA are apparently important in determining Ab pathogenic potential.

The Ig molecule consists of two identical heavy chains and two identical light chains, each of which has a variable (V) and a constant (C) region. Ab specificity was traditionally believed to be determined by the V regions, while the nature of the C region was responsible for Ab effector functions. During maturation of the murine immune response in secondary lymphoid organs, isotype switching can occur through an ordered sequence, generating Abs of different isotypes with presumably the same specificity because of unchanged V region sequences. Through in vitro class switching, it is possible to generate a panel of isotype switched Abs which

Abbreviations: Ag, antigen; Ab, antibody; C, constant; CD, circular dichroism; ds, double stranded; mAb, monoclonal antibody; Sd, distorted β sheet; SLE, systemic lupus erythematosus; SPR, surface plasmon resonance; Sr, regular β sheet; ss, single stranded; Trp, tryptophan; Turn, turns; Unrd, unordered; V, variable; VDJ, variable-joining-diversity.

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share the identical V region of the original Ab (Iglesias-Ussel et al., 2009).

In recent years, studies from several laboratories have demonstrated that the specificity and affinity of Abs are properties that may *not* be exclusively determined by the V region (Torres and Casadevall, 2008; Torres et al., 2007a,b, 2005; Dam et al., 2008; Cooper et al., 1993; Pritsch et al., 2000; Tudor et al., 2012). Indeed, secondary structure changes occurring in Abs following antigen (Ag) binding can differ depending on the isotype (Janda and Casadevall, 2010). Furthermore, C regions can confer new properties to IgG molecules as seen by electronic emission spectra and proteolytic capacity (Janda et al., 2010). Therefore, at least in protective Abs the immunoglobulin C region may directly influence Ab–Ag interactions, possibly through alteration of Ab structure.

We recently generated a panel of murine anti-DNA Abs (IgG1, IgG2b, IgG2a, IgG3) which have identical V (variable-joining-diversity, or VDJ) regions. Members of this Ab panel not only differed in their affinity and specificity for nuclear Ags, but in binding to kidney Ags as well (Xia et al., 2012). Moreover, variations in affinity for renal Ags were mirrored in the analysis of specificity for glomeruli, and were associated with significant differences in renal pathogenicity and survival following Ab administration in vivo. These results suggested that the C regions may be directly affecting the interaction between the Abs to DNA and their cognate Ags, and indicate that C region effects on Ab binding are directly relevant to understanding humoral autoimmunity as well.

How the C regions of anti-nuclear Abs affect binding to target Ags, however, remains unclear. The purpose of this study is to explore this observation and understand the relevant mechanisms, by investigating the effects of heavy chain C regions on the secondary structures of anti-nuclear Abs during their interaction with Ags.

2. Materials and methods

2.1. Generation of the PL9-11 isotype panel

As described previously (Xia et al., 2012), the murine IgG1, IgG2b and IgG2a isotype variants were generated from the parent hybridoma clone of PL9-11 (IgG3) by class switching in vitro. All isotypes shared the original PL9-11 heavy and light chain V region, but differed in the identity of the heavy chain C region. The light chain variable-joining (VJ) sequence was published previously (GenBank: X65011.1). V region amino acid sequences are shown below (Trp residues indicated as "W").

Heavy chain VDJ region: EVKLVESGGGLVKPGGSLKLSCAASGFTFS-NYIMF**W**VRQTP AKRLE**W**VANISSGGGNTYYPDSVKGRFTISRDNVM-NILYLQMSSLRSEDTAMYYCARRAYSNYALDF**W**GQGTSVTVS

Light chain VJ region: DIVMTQSPSSLSVSTGDKVTMSCKSSQ-SLLNSRNQKNYLA**W**YQ QKP**W**QPPKLLIYGASTRKSGVPDRFTGSGSG-TDFTLTISSVQTEDLAIYYC

(from beginning to end, underlined fragments are CDR1, CDR2 and CDR3, respectively).

To purify the mAbs, hybridoma cells were grown in a two-compartment tissue culture flask (Sartorius Co., Edgewood, NY). Supernatants were collected and passed over a protein A (IgG3, IgG2a) or protein G (IgG1, IgG2b) HiTrap column (GE Healthcare, Port Washington, NY). Purified Abs were then carefully normalized to the same Ab concentration by the shared kappa chain using both Western blotting and ELISA.

Coomassie blue staining performed on purified PL9-11 panel antibodies run on a protein gel demonstrated that the antibody preparations were free of significant histone contamination (data not shown).

2.2. Preparation of Ags

dsDNA was obtained from plasmid DNA grown in bacterial cells (which do not contain histones), by digesting with *XmnI*. Single stranded (ss) DNA was prepared by boiling dsDNA at 95 °C for 5 min, followed by immediate cooling on ice. Histones H2A and H2B (DNA-free) were purchased from Roche (Pleasanton, CA). ssDNA, dsDNA, histone H2A and 2B all bound to the parent IgG3 mAb by ELISA and/or Western blot (Xia et al., 2012).

2.3. SPR analysis

The affinity of the PL9-11 Abs for histone 2A and 2B was determined by SPR analysis using a Biacore 3000 instrument (Biacore, Piscataway, NJ). Ab concentration was titrated from 0–200 nM in running buffer (pH 7.4, 20 mM KPO4, 130 mM KCl, 3.4 mM EDTA and 0.005% Tween 20). Histones 2A or 2B (5 nM) were immobilized on a CM sensor chip (GE Healthcare). The Abs were injected over the chip at a flow rate of 30 μl/min, with 280 s for contact time and 350 s for dissociation. All responses were normalized and expressed relative to the baseline defined by the running buffer. The simple Langmuir model ($A + B \leftrightarrow AB$) was used for calculating the binding kinetics of the Ab–Ag complex. Association (K_a) and dissociation (K_d) values were calculated using the Biacore evaluation software 1.1 (Hedberg et al., 2011).

2.4. Trp fluorescence emission

The FluoroMax-3 spectrofluorometer (HORIBA Jobin Yvon, Stanmore Middlesex, UK) was utilized to measure the intensity of fluorescence at a wavelength range of 295–450 nm. The Ab panel, Ags (dsDNA, ssDNA, histone 2A and 2B), and their complexes were prepared at room temperature by mixing the two components 30 min before recording the spectra. All the Ags and Abs were mixed at same final molar concentration of 0.5 μM. Three independent repeats were performed for each experimental condition.

For salt titration, PL9-11 IgG3 and dsDNA (0.5 μM) were solubilized in a NaCl solution that had a concentration of 25, 50, 100, 200, and 400 mM. The final data was recorded after subtracting the spectra of the NaCl solution alone.

2.5. CD spectroscopy

CD spectroscopy was carried out using a Jasco spectrophotometer (JASCO, Easton, MD) (Janda and Casadevall, 2010), with a 350 μl rectangular quartz cuvette for the samples. ssDNA and dsDNA were prepared at final concentrations of 2.5, 5, 10, 20, 40 and 80 nM in PBS. Histones H2A and H2B were used at 80, 90, 100, 110, 120 and 130 nM. Ab concentrations were kept constant at 80 nm. Data were measured from a range of 240 nm to 190 nm. After converting machine units θ_{obs} (millidegrees) to delta epsilon ($\Delta\varepsilon$), the secondary structure prediction was done by using the CONTIN/LL program in the CDPro suite (Janda and Casadevall, 2010). The reference set of protein provided the structures of regular α helix (Hr), distorted α helix (Hd), regular β sheet (Sr), distorted β sheet (Sd), turns (Turn), and unordered (Unrd).

2.6. Control proteins

Murine mAb isotype controls were obtained from Southern Biotech (Birmingham, AL): IgG3, B10 (Ag unknown); IgG1, 15H6 (T-2 mycotoxin); IgG2b, A-1 (chicken IgA); IgG2a, HOPC-1 (unknown). All of the isotype controls were verified not to bind to dsDNA.