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Antibody–Nucleic Acid Complexes. Conformational and Base Specificities Associated with Spontaneously Occurring Poly- and Monoclonal Anti-DNA Antibodies from Autoimmune Mice†

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ABSTRACT: An enzyme-linked immunosorbent assay (ELISA) was developed to characterize spontaneously occurring, mono- and polyclonal anti-DNA antibodies. The assay consists of adsorbing single- (ss) and double-stranded (ds) DNA and various nucleoside–bovine serum albumin conjugates (e.g., A–, G–BSA, etc.) to microtiter wells and assesses the ability of various antibodies to bind to these immobilized antigens. The conformational and base specificity of two monoclonal antibodies (designated MRss-1 BWds-3) was examined in this manner. The exclusive binding of MRss-1 to ssDNA and guanosine–BSA (G–BSA) confirms our previous findings [Munns, T. W., Liszewski, M. K., Tellam, J. T., Ebling, F. M., & Hahn, B. H. (1982) *Biochemistry 21*, 2929–2936] that this antibody recognizes single-stranded nucleic acids by virtue of their guanine content. The extensive binding of BWds-3 to dsDNA, its limited binding to ssDNA, and complete absence of binding to nucleoside–BSA antigens implied a double-stranded conformational specificity. Further, competitive studies with naturally occurring and synthetic alternating copolymers indicated that BWds-3 preferentially recognized the native dsDNA antigens. ELISA analysis of the spontaneously occurring, polyclonal anti-DNA antibodies from MRL/lpr and NZB/NZW-F1 mice revealed that the majority of anti-ssDNA antibodies bound to nucleoside–BSA conjugates. Anti-G antibodies were most prominent in both strains of mice, yet lesser and more variable quantities of anti-A, -C, -U, and -T antibodies were also detected. Preadsorption of serum with G–BSA/Sepharose resulted in the complete removal of anti-G antibodies and a 60% reduction in anti-ssDNA antibodies. Anti-ssDNA antibodies were completely removed by preadsorption of serum with a mixture of A–, G–, C–, and T–BSA/Sepharose. Anti-dsDNA antibodies accounted for approximately 30 and 60% of the total antibody population in the serums of MRL/lpr and NZB/NZW-F1 mice, respectively. Last, time–course studies with five individual MRL/lpr mice revealed that the appearance of all anti-DNA antibodies in their serum was coincident and occurred in all animals at the age of 10–11 weeks.

Various strains of mice, particularly NZB/NZW-F1 and MRL/lpr, have been used extensively as experimental models of human systemic lupus erythematosus (SLE)[1] (Seegal et al., 1969; Andrews et al., 1978). The spontaneous appearance of anti-DNA antibodies in the serum of these mice represents one of the most prominent features of this autoimmune disease. Collectively, such serums possess a multiple of anti-DNA antibodies with diverse antigenic specificities (Tan, 1982; Hahn et al., 1980; Marion et al., 1982; Rauch et al., 1982; Eilat et al., 1980). Specificity is frequently defined by the ability of an antibody to recognize either single- (ss) or double-stranded (ds) nucleic acid antigens (Hahn et al., 1980). Antigenic subsets of anti-DNA antibodies have been reported and include individual bases (Munns et al., 1982a), nucleosides (Weisbart et al., 1982, 1983; Munns et al., 1982b), oligonucleotide sequences (Lee et al., 1981), Z and B configurations of dsDNA (Lafer et al., 1981a, 1983), and sugar–phosphate "backbone" structures (Stollar, 1975). The latter appear to recognize both ss and ds conformations to varying degrees and are believed to be specific for either sugar–phosphate backbones and/or portions of base structures exposed at the surface of dsDNA sequences in Z configurations (Lafer et al., 1983).

Investigations designed to evaluate antibody specificity frequently rely upon various competition–inhibition studies. For example, Hahn et al. (1980) classified various monoclonal preparations into anti-ss DNA and anti-dsDNA antibody categories on the basis of inhibiting their binding to [^{125}I]DNA with defined, unlabeled ss- and dsDNA competitors. Utilizing other competition assays and direct binding studies with nucleoside–BSA/Sepharose adsorbents, we recently determined that one of the above monoclonal antibodies (designated MRss-1) recognized ssDNA by virtue of the antigen's content of guanine residues, i.e., MRss-1 bound to ssDNA–agarose and G–BSA/Sepharose but not to A–, C–, U–, or T–BSA/Sepharose adsorbents (Munns et al., 1982a). Most recently,

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[1] Abbreviations: SLE, systemic lupus erythematosus; ds- and ssDNA, double- and single-stranded DNA; ELISA, enzyme-linked immunosorbent assay; A, adenosine; m^6A, N^6-methyl-A; G, guanosine; m_2^2G, N^2,N^2-dimethyl-G; m^7G, 7-methyl-G; C, cytidine; m^5C, 5-methyl-C; U, uridine; m^5U, 5-methyl-U or ribothymidine; BSA, bovine serum albumin; A–BSA, G–BSA, etc., nucleosides covalently linked to BSA; A–BSA/Sepharose, G–BSA/Sepharose, etc., nucleoside–BSA conjugates coupled to Sepharose; PO_4/NaCl, phosphate-buffered saline; Tris, tris(hydroxymethyl)aminomethane; Tris/NaCl, Tris-buffered saline; IgG, immunoglobulin G.

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Weisbart et al. (1982, 1983) and Munns et al. (1982b) employed immobilized nucleoside–BSA conjugates in ELISA to provide evidence that a significant population of antibodies in the serum of humans possessing various autoimmune disorders recognizes specific nucleosides. Whereas Weisbart et al. (1982a, 1983) demonstrated that antibodies specific for G and/or C were prominent in SLE, Munns et al. (1982b) detected antibodies with apparent specificities for both unmethylated (A, G, C, U, and T) and two methylated nucleosides (m_2^2G and m^7G).

In view of these observations, together with a quantitative ELISA system (Munns et al, 1984), we examined whether similar specificities existed in SLE-like murine model systems. Evidence is presented that indicates the feasibility of ELISA to rapidly detect and evaluate the conformational and/or base specificities of both mono- and polyclonal anti-DNA antibodies. Such an examination of polyclonal anti-DNA antibodies in two murine systems (NZB/NZW-F1 and MRL) led to the conclusion that the majority of determinants present in ssDNA reflect individual bases and, in particular, guanine.

Materials and Methods

Materials. A variety of polynucleotides were used in the present investigation and unless otherwise stated were purchased from Sigma Chemical Co. (St. Louis, MO) and/or P-L Biochemicals (Milwaukee, WI). Single-stranded homopolymers included polyadenylate [poly(A)], polycytidylate [poly(C)], polyguanidylate [poly(G)], and polythymidylate [poly(T)]. Double-stranded DNA alternating copolymers included poly(dA-dT)·poly(dA-dT), poly(dG-dm^5C)·poly(dG-dm^5C), and poly(dG-dC)·poly(dG-dC). The genomes of two single-stranded (ss), circular DNAs were used as a source of ssDNA. Ultrapure, naturally occurring double-stranded (ds) DNAs included those obtained from *E. coli* B, λ bacteriophage, human placenta, and calf thymus (Sigma and P-L Biochemicals).

Various nucleosides including adenosine (A), cytidine (C), guanosine (G), ribothymidine (T), and uridine (U) were coupled to bovine serum albumin (BSA) (Munns et al., 1984) and the resulting conjugates A–BSA, etc. in turn coupled to Sepharose (i.e., A–BSA/Sepharose). Other adsorbents included ssDNA–agarose (Bethesda Research Labs) and DEAE-Sephacel (Pharmacia), the latter used to isolate IgG fractions (Fahey & Horbett, 1959).

Enzyme-Linked Immunoadsorbent Assays (ELISA). Most of the reagents associated with ELISA such as microtiter plates, enzymes, nucleoside–BSA conjugates, etc. are described in the preceding paper (Munns et al., 1984). Additional reagents included both unlabeled and labeled forms of fd and λ DNA ([^{3}H]DNAs from Miles and Bethesda Research Laboratories) and a [^{3}H]IgG fraction of mouse anti-m^7G antibodies (polyclonal) previously labeled with [^{3}H]$NaBH_4$ as described by Tack et al. (1980).

While the [^{3}H]IgG was used to quantitate the nanograms of mouse IgG bound to microtiter wells (see below), fd and λ DNAs were employed as immobilized antigens to detect anti-ssDNA and anti-dsDNA antibodies. Both DNAs were dissolved in PO_4/NaCl (10 mM PO_4, 150 mM NaCl, pH 7.4) to a concentration of 100 μg/mL and these solutions pipetted into individual wells of microtiter plates (0.15 mL/well). After an overnight incubation period, plates were inverted to remove unadsorbed antigen and washed twice with PO_4/NaCl containing 0.05% Tween-20. Whereas nucleoside–BSA antigens and fd DNA were incubated with untreated wells, λ DNA was incubated in wells precoated with poly-L-lysine (Sigma). Pretreatment consisted of incubating 0.15 mL of poly-L-lysine (50 μg/mL in H_2O) in individual wells for 2 h prior to inversion, washing (2× with H_2O), and reincubating with λ DNA as described above. Identical adsorptions conducted with ^{3}H-labeled fd and λ DNA (specific activity 1×10^6 cpm/μg) indicated that these DNAs were adsorbed to an extent of 18 ± 2.5 (fd) and 38 ± 7 (λ) ng/well.

To quantitate ELISA in terms of the nanograms of IgG bound, various dilutions of a [^{3}H]IgG fraction (specific activity 0.9×10^3 cpm/ng) containing anti-m^7G antibodies were incubated with wells coated with m^7G–BSA antigen. Wells were subsequently processed for determination of (i) absorbance (A_{405}) with alkaline phosphatase coupled, goat anti-mouse IgG and (ii) retained radioactivity. Absorbance, graphed as a function of retained radioactivity (actually nanograms of IgG bound on the basis of specific activity), provided the requisite information to convert absorbance measurements into nanograms of IgG bound. All aspects of this quantitation procedure have been described in detail (Munns et al., 1984).

Mice. Female NZB/NZW-F1 hybrids were bred from stock colonies of NZB/B1 and NZW mouse strains maintained at the animal facility at Washington University. MRL/lpr breeding pairs were obtained from Dr. E. Murphy (Jackson Laboratory, Bar Harbor, ME). F1 generations of MRL/lpr and NZB/NZW-F1 hybrids were used as donors of spleen cells for production of cloned hybrid cells secreting monoclonal anti-DNA antibodies (Hahn et al., 1980). Serums from F2 generations of MRL/lpr mice were collected at monthly intervals and processed for determination of anti-DNA antibodies as evaluated via the Farr assay (Riley et al., 1979) and by the ELISA described herein.

Monoclonal Anti-DNA Antibodies. Procedures relating to the production of monoclonal anti-DNA antibodies from autoimmune unimmunized mice have been described (Hahn et al., 1980, 1983; Munns et al., 1982a, 1984) with respect to their selection (ELISA and Farr assay), propagation (pristane-treated Balb/c mice), and isotype (protein A/Sepharose and/or anti-isotypic antibody reagents). Two monoclonal antibodies designated MRss-1 (IgG2a,κ) and BWds-3 (IgG2b,κ) were employed in the present study. Their designations reflect origin (MRL or B/W-F1), conformational specificity (ss- or dsDNA), and order of characterization.

ELISA: Characterization of Mono- and Polyclonal Anti-DNA Antibodies. Antibody populations were characterized by their ability to bind with various immobilized antigens. Antigens immobilized to evaluate conformational specificity included fd DNA (ss, circular) and λ DNA (ds, linear). Nucleosides conjugated to BSA via their ribose moiety (Erlanger & Beiser, 1964; Munns & Liszewski, 1980) were employed to ascertain individual base specificities associated with anti-ssDNA antibodies. Nucleosides conjugated to BSA included A, N^6-methyl-A (m^6A), G, N^2,N^2-dimethyl-G (m_2^2G), 7-methyl-G (m^7G), C, 5-methyl-C (m^5C), U, and T. Each conjugate possessed between 15 and 20 nucleosides (haptens) per BSA molecule. All aspects of the ELISA, immobilization of antigens, incubation conditions, absorbance measurements, etc. have been described in detail (Munns et al., 1984).

Competition and Preadsorption Studies. To further define the specificity of spontaneously occurring, poly- and monoclonal anti-DNA antibodies, antiserum and various IgG fractions were diluted in PO_4/NaCl containing 2 mg/mL BSA (PO_4/NaCl/BSA) and preincubated with various quantities of defined nucleic acid substrates for 30 min at 37 °C prior to incubation in antigen-coated microtiter wells. Competing soluble substrates included a variety of naturally occurring DNAs and synthetic homopolymers and alternating co-

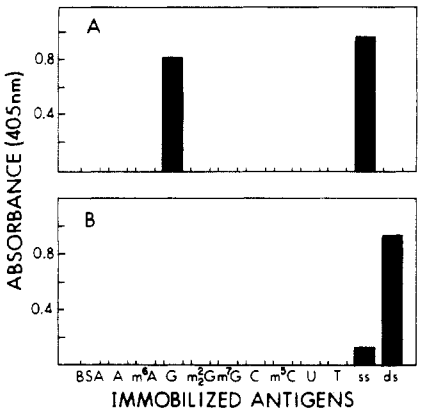

FIGURE 1: IgG fractions containing MRss-1 (panel A) and BWds-3 (panel B) were diluted 250-fold in PO_4/NaCl/BSA and 0.15-mL aliquots incubated in individual microtiter wells containing immobilized antigen. Immobilized antigens included BSA, A, m^6A, G–BSA, etc. and ss- and dsDNA. Conditions associated with these incubations, absorbance measurements (A_{405}), etc. are described under Materials and Methods.

polymers (listed above). At the conclusion of this preincubation period, aliquots were subjected to ELISA for further definition of antibody binding capabilities to immobilized antigens.

Restriction fragments of λ (dsDNA), φX174, and fd (ssDNA), generated in the presence of *Hae*III restriction endonuclease, were also used as competing antigens in the above assay. DNA was hydrolyzed by using conditions previously described by Blakely et al. (1977). Fragments were isolated free of *Hae*III by phenol extraction and ethanol precipitation. Electrophoresis in agarose (1.0%) and acrylamide (5%) gels (Blakely et al., 1977) revealed (i) the presence of multiple DNA fragments for λ (25), φX174, and fd (8–12) DNAs and (ii) the absence of significant quantities of intact DNA.

Additionally, preadsorption investigations were conducted in an attempt to remove specific populations of antibodies prior to assessment via ELISA. Antiserum previously diluted in PO_4/NaCl/BSA was incubated with various nucleoside–BSA/Sepharose and ssDNA–agarose adsorbents (bed volume of all adsorbents 25 μL) for 30 min at 37 °C prior to a centrifugation step (1 min at 2000*g*) for removal of adsorbent-bound antibody. Antibody activities remaining in the supernatant were assessed via ELISA.

Results

ELISA: Characterization of Monoclonal Anti-DNA Antibodies. Recently, we reported that a murine monoclonal antibody designated MRss-1 was specific for guanine-containing single-stranded nucleic acids (Munns et al., 1982). Base specificity was determined by evaluating the ability of [^{3}H]MRss-1 to bind to (i) ssDNA–agarose in the presence of unlabeled sequence-defined nucleic acids, oligonucleotides, and mononucleotides and (ii) nucleoside–BSA/Sepharose adsorbents, e.g., G–, C–BSA/Sepharose, etc. In view of the adaptability of ELISA in characterizing polyclonal anti-nucleoside antibodies (Munns et al., 1984), we reevaluated the specificity of MRss-1 by asssessing its ability to bind with a broad spectrum of immobilized antigens (e.g., nucleoside–BSA conjugates and ss- and dsDNA). Additionally, we assessed in an identical manner a second murine monoclonal antibody designated BWds-3. Selected on the basis of an ELISA employing immobilized λ DNA, BWds-3 was suspected to be specific for dsDNA on the basis of various competition studies (see below).

The data obtained by reexamining these monoclonal antibodies via ELISA are presented in Figure 1 and indicate that MRss-1 (panel A) binds to both G–BSA and ssDNA antigens but not to other nucleoside–BSA conjugates, BSA, or dsDNA. In contrast, BWds-3 antibody (panel B) was bound primarily by dsDNA and slightly by ssDNA but not by any of the nucleoside–BSA conjugates or unconjugated BSA. We suspect that the slight cross-reactivity of BWds-3 with ssDNA (fd DNA) can be attributed to the existence of ds hairpin regions reportedly present in this nucleic acid (Shen et al., 1979; Wells et al., 1980).

While the above results were predictable and in accord with our previous findings (Hanh et al., 1980; Munns et al., 1982a), they illustrate the simplicity, speed, and accuracy with which ELISA methodology can be used to detect anti-DNA antibodies as well as determine their conformational specificities. Further, and as illustrated by the MRss-1 binding profile, both conformational (ssDNA) and base (guanine) specificity was determined by ELISA. Also to be stressed is the finding that MRss-1 does not bind to immobilized dsDNA. This finding indicates that immobilized dsDNA does not possess significant amounts of ss regions, i.e., an ideal immobilized antigen for detecting anti-dsDNA.

To obtain additional information regarding the conformational specificity of BWds-3 and MRss-1, we modified the ELISA by preincubating well-defined nucleic acid substrates with these monoclonal antibodies prior to assessing their binding to immobilized DNA. Whereas poly(G), φX174, and fd DNA were potent inhibitors of MRss-1 binding to immobilized ssDNA, other homopolymers and all dsDNA competitors (alternating copolymers, λ, *E. coli*, thymus, and placental DNA) were ineffective in this regard [data not shown; see Munns et al. (1982)]. Again, the inability of these dsDNA substrates to inhibit MRss-1 binding to ssDNA implies that all naturally occurring DNAs as well as the alternating copolymers poly(dG-dC)·poly(dG-dC) and poly(dG-dm^{5}C)·poly(dG-dm^{5}C) lack significant quantities of guanine-containing, single-stranded regions.

The results of the competition assays associated with BWds-3 are presented in Figure 2A. These data revealed that while various homopolymers were ineffective as competitors, all of the dsDNA substrates employed inhibited BWds-3 binding to immobilized dsDNA. However, naturally occurring dsDNAs were considerably more effective as competitors when compared with the synthetic alternating copolymers, the latter requiring 50- to 100-fold more DNA to achieve the same levels of inhibition.

Also interesting was the finding that both φX174 and fd DNAs effectively inhibited the binding of BWds-3 to immobilized λ DNA (Figure 2B). To prove the existence of ds regions in these ssDNAs, both nucleic acids were digested with *Hae*III and the resulting fragments assessed in competition assays. These results also presented in Figure 2B revealed that prior digestion of φX174 and fd DNA with *Hae*III resulted in a significant loss in their ability to inhibit BWds-3 binding. In contrast, the restriction fragments of a dsDNA (λ) were without effect. These results are in accord with the previous findings of Shen et al. (1979) and Blakely et al. (1977), which indicate that both φX174 and fd DNAs contain ds regions within their ss genomes.

ELISA: Characterization of Polyclonal Anti-DNA Antibody Populations in MRL and B/W Mice. While the above data demonstrate the applicability of ELISA for characterizing monoclonal anti-DNA antibodies, such antibodies are not representative of the total anti-DNA antibody population in

Table I: Binding of Spontaneously Occurring, Polyclonal Anti-DNA Antibodies to Immobilized Nucleoside–BSA and Nucleic Acid Antigens[a]

	IgG bound (ng) to antigen-coated wells for antigens							
mice[b]	BSA	A	G	C	U	T	ss	ds
MRL-1	ND[c]	1.9	5.3	0.6	1.4	1.9	8.5	3.0
MRL-2	ND	1.5	4.7	ND	ND	1.1	7.2	3.0
MRL-3	ND	1.4	8.0	0.7	0.4	1.0	9.2	2.0
MRL-4	ND	2.0	4.2	ND	1.6	1.5	8.7	1.8
MRL-5	ND	1.5	3.4	ND	1.2	2.0	6.2	2.8
MRL-6	ND	ND	ND	ND	ND	ND	ND	ND
B/W-1	ND	0.8	2.7	ND	ND	0.8	3.4	4.0
B/W-2	ND	1.2	2.1	ND	1.0	ND	2.9	4.8
B/W-3	ND	0.6	2.4	0.5	0.9	0.9	3.2	6.1
B/W-4	ND	0.8	3.5	ND	ND	0.6	4.7	6.2
B/W-5	ND	ND	ND	ND	ND	ND	ND	ND
Balb/c-1	ND	ND	ND	ND	ND	ND	ND	ND
Balb/c-2	ND	ND	ND	ND	ND	ND	ND	ND
C57-1	ND	ND	ND	ND	ND	ND	ND	ND
C57-2	ND	ND	ND	ND	ND	ND	ND	ND

[a] Serums from individual MRL and B/W mice were diluted 150-fold with PO_4/NaCl/BSA (i.e., 1.0-μL equivalent/assay) and processed via ELISA as described under Materials and Methods. Variation of the mean value of three independent assays was less than 16%. Variation was less than 7% for values exceeding 2 ng of bound antibody. [b] Mice: MRL-1, 22-week-old male; MRL-2 and -3, 22-week-old females; MRL-4, 14-week-old male; MRL-5, 14-week-old female; MRL-6, 6-week-old female; B/W-1, -2, -3, and -4, all 32-week-old females; B/W-5, 4-week-old female. Balb/c-1 and -2 and C57-1 and -2 are pooled serums from five male and female mice (6–8 months old), respectively. Pooled serums were 10-fold more concentrated than MRL and B/W serums (i.e., 2-μL equivalent per animal per assay). [c] ND, not detected or less than 0.3 ng of IgG bound.

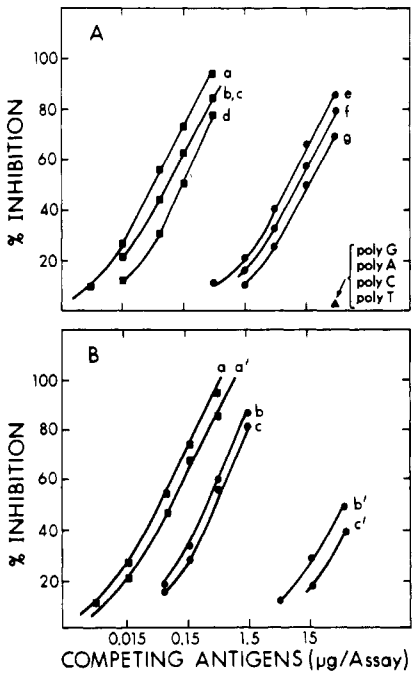

FIGURE 2: Specificity of BWds-3 as evaluated by competition–inhibition assays employing immobilized λ DNA. Aliquots of an IgG fraction containing BWds-3 and yielding an A_{405} of 0.9 (see legend of Figure 1) were preincubated with various quantities of competing nucleic acids (30 min, 37 °C) prior to reincubation (30 min, 37 °C) in microtiter wells containing immobilized λ DNA. In panel A, competing soluble antigens included λ (a), calf thymus (b), human placental (c), and *E. coli* B (d) DNA while e, f, and g represent poly(dG-dm^{5}C)·poly(dG-dm^{5}C), poly(dG-dC)·poly(dG-dC), and poly(dA-dT)·poly(dA-dT), respectively. The homopolynucleotides of G, A, C, and T are represented by the closed triangle. In panel B, competing soluble antigens included intact λ (a), fd (b), and φX174 (c) DNA and their respective fragments (a′, b′, and c′) after *Hae*III digestion. Antibody binding to immobilized λ DNA was measured by ELISA as described under Materials and Methods. Data are presented as the mean of three independent experiments with individual values varying less than 16% of the mean.

the serum of autoimmune mice. To obtain an overview of the entire anti-DNA antibody population, serum from mice possessing significant levels of anti-DNA antibodies (as determined by the Farr assay; Han et al., 1980; Ebling & Hahn, 1979) was subjected to analysis via ELISA. These results are presented in Table I and include data obtained from strains of autoimmune (MRL and B/W) and normal (Balb/c and C-57) mice. Most pronounced in the MRL system were antibodies with apparent specificities directed almost entirely to G–BSA, m_2^2G–BSA, and ssDNA antigens, with smaller, yet detectable quantities of additional antibodies binding to A–, C–, U–, and T–BSA and dsDNA. While uncertain, we attribute the binding to m_2^2G–BSA antigen to anti-G antibody in view of our previous findings that experimentally induced, polyclonal anti-G antibody cross-reacts extensively with m_2^2G (Munns et al., 1984). Additional support of such cross-reactivity stems from the observation that adsorption of MRL serum with G–BSA/Sepharose removes both G and m_2^2G binding activities (for additional details see Figure 3).

B/W serum, in contrast, possessed significantly higher levels of anti-dsDNA antibodies relative to those binding to G–BSA and ssDNA antigens (Table I). Again, small and variable quantities of antibodies binding to other nucleoside–BSA antigens were also observed. Also shown in Table I are our findings that pooled serums from Balb/c and C57 mice (i.e., strain not predisposed toward autoimmune disease) demonstrated a complete lack of anti-DNA antibody binding activity as evaluated by ELISA. Further, these control serums were incubated in 10-fold excess relative to MRL and B/W serums. Similar results were obtained from the serums of younger MRL and B/W mice (see legend to Table I).

Specificity of Polyclonal Anti-ssDNA Antibodies. While Table I indicates that autoimmune mice possess relatively high levels of antibodies that recognize G–BSA, ssDNA, and dsDNA antigens, it does not provide sufficient information to determine if (i) anti-G and anti-ssDNA antibodies reflect the same population or if (ii) antibody binding to individual nucleoside–BSA conjugates represents unique base specificities or cross-reactivities. To obtain this information, we preincubated antiserum with various nucleoside–BSA/Sepharose and ssDNA–agarose adsorbents and reassessed antibody binding activity in the supernatants upon removal of adsorbent-bound antibodies via centrifugation. The results from an MRL serum

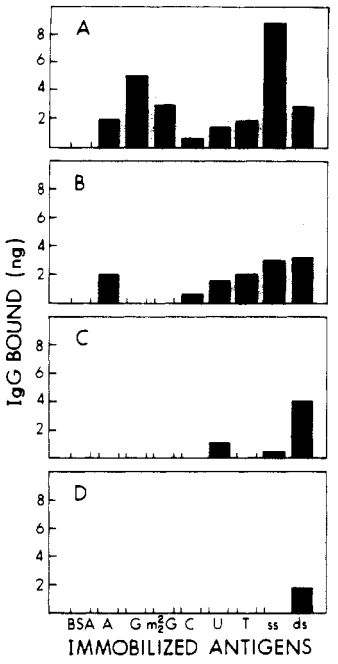

FIGURE 3: Adsorption of specific antibody populations with nucleoside–BSA/Sepharose and ssDNA–agarose. Serum from an MRL/lpr mouse (MRL-1, see legend to Table I) was diluted 150-fold with PO_4/NaCl/BSA and incubated in the presence of nucleoside–BSA/Sepharose or ssDNA–agarose (bed volume 50 μL) for 30 min at 37 °C. Adsorbent and bound antibody were removed by centrifugation and the supernatant was assessed via ELISA for antibody binding activities. Additional details regarding these protocols appear under Materials and Methods. Serum was preadsorbed with BSA/Sepharose (panel A), with G–BSA/Sepharose (panel B), with a mixture of A–, G–, C–, and T–BSA/Sepharose (panel C), and with ssDNA–agarose (panel D).

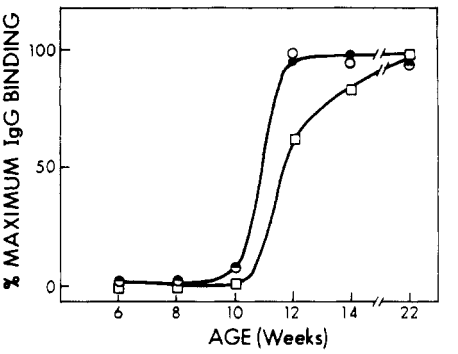

FIGURE 4: Time–course study regarding the appearance of specific antibody populations in the serum of a single MRL/lpr mouse (MRL-1, see legend to Table I). Serum was obtained at biweekly intervals and processed via ELISA as described in the legend of Figure 3 and under Materials and Methods. Data were plotted as the percent of the maximum amount of antibody bound to immobilized G–BSA (5.2 ng, open circles), to ssDNA (8.7 ng, closed circles), and to dsDNA (3.1 ng, open squares). Almost identical results were obtained with two other MRL mice (MRL-2 and -4). In all experiments variation was less than 10% of the mean values for values exceeding 2.0 ng.

are presented in Figure 3 (in part) and revealed the following information. First, preadsorption with G–BSA/Sepharose resulted in the complete removal of anti-G (and m_2^2G) antibodies, approximately 60% removal of the anti-ssDNA antibodies, and little or no significant removal of anti-A, -U, -T, and dsDNA antibodies (compare parts A and B of Figure 4). Second, preadsorption with T–SBA/Sepharose resulted in the complete removal of anti-T antibodies and about 25% removal of the anti-U and anti-ssDNA antibodies but was ineffective in removing anti-G, -m_2^2G, -A, -U, and dsDNA antibodies (data not shown). Third, adsorption with a combination of A–, G–, C–, and T–BSA/Sepharose adsorbents resulted in complete removal of all activities except anti-dsDNA antibodies (unaffected) and anti-U antibodies (30% reduction, compare parts A and C of Figure 3). Last, preadsorption with ssDNA–agarose resulted in the removal of all antibody binding activities except anti-dsDNA antibodies, which were reduced by 60% (compare parts A and D of Figure 3). Although we attribute the reduction of anti-dsDNA antibodies to small quantities of dsDNA in ssDNA–agarose, the possibility exists that some anti-dsDNA antibodies cross-react with ssDNA.

Collectively, these data indicate that anti-ssDNA antibodies reflect to a large extent the sum of antibodies with apparent specificities directed toward individual base components present in DNA. This observation is also supported by the data in Table I, which indicate that the sum of the nanograms of antibody bound to individual nucleoside–BSA substrates (excluding m_2^2G– and U–BSA) approximates the total nanograms of antibody binding to immobilized ssDNA. Other preadsorption studies not presented revealed similar findings when other MRL and B/W serums underwent identical analysis. Last, the inability of unconjugated BSA/Sepharose to alter the binding profiles of these polyclonal anti-DNA antibodies revealed that adsorptions with nucleoside–BSA/Sepharose adsorbents were immunospecific.

Time–Course Studies. To complete our investigations regarding the specificity of anti-DNA antibodies, we monitored the time–course of appearance of anti-DNA antibodies in the serum of four individual MRL mice. Representative of our findings are the results from a single mouse, which are presented in Figure 4, with regard to the appearance of anti-G, anti-ssDNA, and anti-dsDNA antibodies. Whereas the quantity of these antibodies remains insignificant during the first 8 weeks, their appearance in serum after 10 weeks was rapid and coincident. Whereas maximum or near-maximum levels of anti-ssDNA antibodies were achieved within an additional 4 weeks, the accumulation of anti-dsDNA antibodies appeared to lag that of anti-ssDNA antibodies. As expected, increased levels of anti-G antibodies (as well as the other less prevalent anti-nucleoside antibodies not illustrated) paralleled increases in anti-ssDNA antibody. A similar study using B/W mice was not conducted.

Discussion

The results presented herein indicate the feasibility of ELISA for detecting anti-DNA antibodies in the serum of mice possessing autoimmune disorders. Such as assay coupled with a broad spectrum of immobilized antigens representative of ss- and dsDNA and the bases present in nucleic acids permits assessment of both conformational and base specificities (Figures 1 and 2). This was demonstrated not only for two monoclonal preparations but also for various heterogeneous populations of spontaneously occurring polyclonal anti-DNA antibodies as well (Table I and Figure 3). Although the data obtained with MRss-1 was only confirmatory [see Munns et al. (1982a)], they illustrate the simplicity and speed by which antibodies can be characterized via ELISA in contrast to the more laborious competition–inhibition studies. Besides the advantage mentioned above, the ELISA does not depend upon the use of radioactive antigens as required by the more commonly employed Farr assay (Wold et al., 1968; Riley et al., 1979). Because of these findings we are now examining the usefulness of ELISA in detecting and characterizing anti-DNA antibodies in the serum of patients suspected of possessing various autoimmune disorders (Tan, 1982). Our preliminary findings in this regard reveal that the bulk of anti-DNA an-

tibodies is specific for single-stranded nucleic acids, yet individual base specificities are more diverse than those reported here in the murine systems. These findings are similar to those of Weisbart et al. (1983). Most notable in the latter investigation was the finding that SLE and rheumatoid arthritis (RA) patients possessed antibody activities primarily directed toward G and C (SLE) and A (RA).

The results regarding the binding of BWds-3 to immobilized ss- and dsDNA suggest that this monoclonal antibody is specific for dsDNA. We suspect that its ability to bind minimally to immobilized fd DNA reflects the existence of small dsDNA regions reportedly present in the ss antigen (Shen et al., 1979; Wells et al., 1982). This rationale receives considerable support from our findings that *Hae*III digestions of ϕX174 and fd DNAs significantly reduce their ability to compete with immobilized dsDNA for BWds-3 binding. The inability of BWds-3 to bind with immobilized nucleoside–BSA conjugates further suggests that ordered deoxyribose and/or phosphate backbone structures of dsDNA represent major determinant(s). BWds-3 preferential recognition of naturally occurring dsDNA relative to synthetic alternating copolymers suggests that the latter substrates possess conformations distinctly different from the former.

It is important to stress, however, that while competition and direct binding assays provide insight into the nature of the antigenic determinant, they do not provide unequivocal evidence as to the identity of the autoantigen. Thus, as suggested by Lafer et al. (1981b), it is conceivable that other molecules of similar structure to bases and/or sugar–phosphate backbones (e.g., heterocyclic drugs, complex polysaccharides, phospholipids, etc.) are responsible for eliciting the production of autoantibodies that cross-react with DNA.

In an attempt to identify the antigen determinant(s) within ssDNA that is (are) responsible for anti-ssDNA antibody binding, mouse serums were preadsorbed with various nucleoside–BSA/Sepharose adsorbents prior to analysis of the supernatants via ELISA. The results of these experiments (Figure 3) indicate that the bases within ssDNA and especially guanine appear to represent the major antigenic determinants. This assumption is based upon numerous observations. First, the sum of the quantity of antibody bound to individual nucleoside–BSA antigens (excess) approximates the quantity of antibody bound to immobilized ssDNA (excess). Second, and most important, preadsorption of serum with a nucleoside-containing adsorbent, e.g., G–BSA/Sepharose, (i) does not affect antibody binding to other nucleoside–BSA antigens, (ii) completely abolishes antibody binding to G–BSA antigen, and (iii) reduces proportionately the amount of anti-ssDNA antibody binding to ssDNA (see Figure 3). These data indicate that a stoichiometric relationship exists between the amount of antibody binding to nucleoside–BSA conjugates and ssDNA. Further, such results would not be expected if major determinants present in ssDNA were oligonucleotide sequences and/or sugar–phosphate structures. While it is reasonable to assume that antibodies with such specificities exist, our data suggest that they reflect a minor population of anti-ssDNA antibodies in MRL and B/W mice and/or are not reactive in our ELISA.

As characterized via ELISA, it appears that the majority of anti-ssDNA antibodies in both murine and human SLE preferentially recognize guanine (murine) or guanine and cytosine (human; see Weisbart et al., 1983). While the reason for this narrow specificity remains uncertain, it is of interest in view of the recent findings of Sano & Morimoto (1982) and Sano et al. (1983). These investigators determined that the DNA isolated from immune complexes of serum from SLE patients possessed significantly higher dG and dC content than that contained in human nuclear DNA. Further, they observed that peripheral blood lymphocytes (from SLE patients) synthesized small dG-dC-enriched DNA fragments, which were absent or significantly reduced in normal control lymphocytes. On the basis of these and other results, these authors suggest that such DNA fragments may serve as a primary source of autoantigen for anti-DNA antibody production.

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Registry No. Guanine, 73-40-5.

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β-Globin Gene Family in Murine Erythroleukemia Cells Resides within Two Chromatin Domains Differing in Higher Order Structure†

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ABSTRACT: The β-globin gene family is organized into two distinct chromatin domains which are digested at significantly different rates by DNase I. We have investigated the possibility that this differential DNase I sensitivity is based upon differences in the higher order structure of chromatin. When nuclei are digested under low ionic strength conditions known to unfold higher order chromatin structures, the differential sensitivity is lost. That is, the relatively DNase I resistant domain, containing the transcriptionally inactive embryonic and β-homologous globin genes, becomes sensitive. When chromatin is recondensed with either $MgCl_2$ or NaCl, thus indicating the higher order coiling of the chromatin fiber, the differential sensitivity is restored. Furthermore, the removal of histone H1, known to be essential for stabilization of higher order chromatin structures, results in the loss of differential DNase I sensitivity. In contrast to the DNase I resistant domain, the transcriptionally active adult β-globin genes show no increase in the rate of digestion when chromatin is unfolded, indicating that this domain may exist as an unfolded nucleosomal chain. The data further suggest that this sensitive domain may be depleted of histone H1.

The primary level of nucleosomal organization is the 10 nm in diameter nucleofilament consisting of nucleosome particles connected by short stretches of linker DNA. Numerous levels of chromatin organization exist between the 10-nm filament and the very compact metaphase chromosome [for a review, see Ris & Korenberg (1979) and McGhee & Felsenfeld (1980)]. Much attention has been focused on the 25-30-nm diameter chromatin fiber which is thought to consist of the 10-nm nucleosomal filament coiled into a close-packed helical array or solenoid (Finch & Klug, 1976; Worcel & Benyajati, 1977; Thoma et al., 1979; Butler & Thomas, 1980; Thoma & Koller, 1981). It has been demonstrated that the conversion of the 30-nm solenoid to the 10-nm filament is a reversible process dependent on the monovalent and/or divalent cation concentration (Thoma et al., 1979; Butler & Thomas, 1980) and the ability of oligonucleosomes to re-form native solenoid structures is strongly correlated with the presence of histone H1 (Butler & Thomas, 1980; Thoma & Koller, 1981).

Nucleases have been widely used to probe chromatin structure, and digestions of nuclei with DNase I have demonstrated that active genes are more accessible to nuclease attack compared to inactive genes (Weintraub & Groudine, 1976). Subsequent studies on the globin genes in chicken erythrocytes (Stalder et al., 1980) and the ovalbumin gene family in hen oviducts (Lawson et al., 1982) have shown that the nuclease-sensitive domains extend well beyond gene coding regions and that these DNase I sensitive domains correlate with transcriptional potential. We have previously demonstrated that the β-globin gene complex in murine erythroleukemia (MEL) cells is organized into two distinct chromatin domains on the basis of their DNase I sensitivity (Smith et al., 1984). The actively expressed β-major and β-minor globin genes are organized into a DNase I sensitive domain while the inactive embryonic and β-homologous (βh) globin genes reside in a relatively insensitive chromatin domain.

In this paper, we have examined the relationship between chromatin domains defined by DNase I and their higher order structure. Specifically, we have determined the effect of (i) altering the ionic strength and (ii) removing histone H1 on the DNase I sensitivity of these two unique globin gene chromatin domains. The findings suggest that the inactive embryonic and βh globin genes reside in a chromatin structure which responds to ionic strength in a manner consistent with reversible transitions in the folding of the 25-30-nm chromatin fiber. In contrast, the active adult globin genes reside in an unfolded structure similar to that of the reported 10-nm chromatin filament. These and other findings are discussed in terms of the primary organization of transcriptionally active and inactive genes in chromatin.

Materials and Methods

Cell Culture. Stock cultures of DS19, a subclone of Friend erythroleukemia cell line 745 derived from DBA/2J mice

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