A SINGLE GERMLINE V_H GENE SEGMENT OF NORMAL A/J MICE ENCODES AUTOANTIBODIES CHARACTERISTIC OF SYSTEMIC LUPUS ERYTHEMATOSUS

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Antibodies that bind to nucleic acids, ribonucleoproteins, and cytoskeletal proteins are characteristic of systemic lupus erythematosus (SLE) and several other autoimmune diseases (1). The source of these autoantibodies has been sought by immunochemical, idiotypic, and structural analyses (reviewed in 2), but whether an exogenous antigen or defective immunoregulation accounts for their production is unknown. An important clue about their origin is that anti-DNA mAbs have the frequently observed property of polyreactivity, i.e., the ability to bind to more than one nuclear or cytoplasmic antigen. A polyreactive monoclonal lupus autoantibody can bind to both DNA and cytoskeletal proteins (3), to both DNA and the Sm ribonucleoprotein antigen (4), to several different polynucleotides and phospholipids (5), or to DNA and polypeptides on cell membranes (6). Polyreactivity is also a feature of monoclonal autoantibodies that bind to specific components of different organs of the endocrine system (7). Some mAbs with reactivity against nucleic acid antigens also bind to exogenous antigens like bacterial polysaccharides (8) and organic chemical haptens (9); reviewed in (10). These unusual crossreactions are immunochemically specific: the binding of anti-DNA mAbs to bacterial polysaccharides is inhibited only by polysaccharides with particular sugar linkages (8), and reciprocal inhibition of ligand binding occurs in competition assays performed with DNA and TNP (9).

These kinds of binding specificities for both autoantigens and exogenous antigens may provide additional clues about the origins of autoantibodies. We wondered whether such dual reactivity implies a physiological relationship between autoantibodies and antibodies against exogenous antigens. Diamond and Scharff (11) showed that a mutation in the V_H gene of the myeloma protein S107 (glu 35 replaced by ala) led to a loss of the antibody's phosphorylcholine-binding activity and a gain in its ability to bind to DNA and cardiolipin. Their finding

raises the possibility that autoantibodies arise by mutation of genes that encode antibacterial antibodies.

In the experiments reported here, we tested an alternative hypothesis about the origins of autoantibodies; namely, that unmutated germline V genes expressed by preimmune B cells are a source of autoantibodies. Such autoantibodies could give rise to antibodies against exogenous antigens by mutation and antigen selection. To test this hypothesis, we analyzed the antigen-binding properties of antibodies produced during the course of the immune response of normal mice to the hapten p-azophenyl arsonate (Ars). Antibodies produced by hybridomas that were selected by a DNA probe for the $V_H Id^{CR}$ gene were studied. This gene segment encodes the idiotype termed Id^{CR} (or CRI-A), which dominates the immune response of A/J mice to Ars (12). The development of V_H gene diversity by somatic mutation after Ars immunization has been studied in detail (13), but the antigenic specificities of the preimmune (nonmutated) repertoire of antibodies encoded by the $V_H Id^{CR}$ gene are unknown. We show here that a high proportion of members of the preimmune antibody repertoire of this antihapten system react with DNA and cytoskeletal proteins. Of 26 antibodies encoded by the unmutated $V_H Id^{CR}$ gene, 50% bound to DNA. Furthermore, Ars immunization seemed to divert the autoreactivity of preimmune antibodies to Ars-reactivity. The apparent conversion was probably due to somatic mutation of $V_H Id^{CR}$ followed by specific selection of high-affinity anti-Ars antibodies from a pool of autoreactive precursors.

Materials and Methods

Antigens. The synthetic polynucleotides poly(I) and poly(dT) were purchased from P. L. Biochemicals, Milwaukee, WI, and Collaborative Research, Waltham, MA. Native DNA (nDNA), single-stranded DNA (ssDNA),[1] and RNA were prepared as described previously (14). Ars-KLH and Ars-tyrosine were prepared as described previously (12).

Antibodies. mAbs from unimmunized A/J mice were obtained from hybridomas that were prepared after in vitro stimulation of spleen cells with either LPS or goat anti–mouse IgM. mAbs from immunized A/J mice were obtained from hybridomas prepared after one or two injections of Ars-KLH. H130 is an anti-DNA mAb produced by a hybridoma from an MRL *lpr/lpr* mouse (15); TEPC 183 was purchased from Litton Bionetics, Charleston, SC; and pooled normal mouse immunoglobulin was purchased from Cappel Laboratories, Cochranville, PA.

Screening and Selection of Hybridomas. All of the A/J hybridoma antibodies were derived and structurally characterized in earlier studies. Group I hybridomas were derived by fusion of mitogen-stimulated spleen cells from nonimmune A/J mice, and were selected on the basis of production of mRNA homologous to the $V_H Id^{CR}$ DNA probe as reported (16). Partial sequences of J_H, D, and V_H regions were determined by the method of Kaartinen et al. (17). >2,000 nucleotides of sequence obtained in this way from preimmune hybridomas revealed no somatic mutations (13 and T. Manser, unpublished results).

Group II hybridomas (primary immune response) were obtained in fusions performed within 9 d after single immunizations with Ars-KLH as reported (16). With the exceptions of P65L9-2 and P65L9-3, all hybridomas were isolated on the basis of their production of antibody that binds to a rabbit antiserum which recognizes the strain A major crossreactive idiotype. The two exceptions were selected in a screen that detects mRNA homologous to the $V_H Id^{CR}$ probe (18). Antibodies synthesized by these hybridomas have thus far shown no somatic mutations. A total of ~1,500 nucleotides of mRNA sequence revealed no change from the germline J_H and $V_H Id^{CR}$ gene segments (16). Included in the

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[1] *Abbreviation used in this paper:* ssDNA, single-stranded DNA.

sequenced samples were the most strongly DNA-binding antibodies, P65H3-1 and P6514-1, including the complete V_HId^{CR} CDR-2 region in each case.

Hybridomas in group III were isolated from fusions of A/J spleen cells after two immunizations with Ars. Members of the hvH65 series were selected on the basis of V_HId^{CR} mRNA production as reported, (16, 17), and the others by serological criteria (19–22). Groups IV and V hybridomas produce antibodies that are not encoded by the V_HId^{CR} gene segment and have been described elsewhere (16, 22, 23).

Ligand-binding Assays. mAbs were tested for their ability to bind to ssDNA by means of an ELISA (8). The polynucleotide was diluted to a concentration of 3.0 μg/ml in Tris buffered saline (TBS), and 150 μl of the solution was added to poly-L-lysine–coated plates; after 2 h the plates were washed three times with Tris-buffered saline. The plates were then blocked with poly-L-glutamate and stored at 4°C for at least 12 h. The wells were blocked with phosphate-EDTA buffer containing 1% BSA for 30 min at 23°C before addition of the antibody to the coated wells. The plate was washed with PBS containing 0.1% Tween-20; goat anti–mouse Ig conjugated to alkaline phosphatase was then added, and the plate was incubated overnight at 23°C. Determination of bound alkaline phosphatase was performed as described previously (8). Reaction of the antibodies with Ars was examined by RIA using Ars-BSA on the solid phase (24). The affinity of the reaction was measured by fluorescence quenching (24).

For competitive immunoassays, equal volumes of the mAb and various dilutions of the inhibitor (polynucleotide, Ars-tyrosine, or antiidiotype) were mixed and incubated for 1 h at 37°C, and then at 4°C overnight. The mixtures were tested by ELISA for their residual binding to the plate.

Immunofluorescence Assays. Human fibroblasts (GM 0152 cell line; Institute For Medical Research, Camden, NJ) grown on coverslips were obtained just before confluence and fixed for 5 min in methanol at −20°C. These cells and HEP2 cells (Bion, Park Ridge, IL) were incubated in 40 μl of the antibody, at a concentration of 100 μg/ml, in a humid chamber for 45 min at 20°C. The cells were washed in PBS and then stained with a fluorescein-labeled IgG fraction of a goat anti–mouse Ig serum (Cappel Laboratories) at a 1:20 dilution of the original antibody protein concentration of 10 mg/ml. The preparations were examined with fluorescence epiillumination. Magnification in all photographs is 600 ×.

Results

Binding of V_HId^{CR}-encoded Antibodies to DNA. We studied 44 mAbs that had been obtained from hybridomas of A/J origin (Table I). Of the 37 hybridomas that expressed the V_HId^{CR} gene, 10 were derived from unimmunized A/J mice (group I); 12 were obtained after a single injection of Ars-KLH (group II); and 15 were derived after two injections of Ars-KLH (group III). The hybridomas in groups I–III were selected by screening with either a DNA probe that detects the germline gene V_HId^{CR} or by a serological method that detects the V_HId^{CR} gene product. The two antibodies in group IV were selected by screening hybridomas with a DNA probe for the V_HId^{36-60} gene. Five other antibodies were coded by unrelated V_H genes (group V). Reactions with ssDNA in a solid-phase ELISA were found with 14 antibodies: 7 of 10 in group I, 3 of the 12 in group II, and 4 of 15 in group III (Table I). The 10 anti-DNA antibodies from groups I and II are partially encoded by the nonmutated germline V_HId^{CR} gene (16). None of the anti-DNA antibodies of group I had detectable binding to Ars. The three group II anti-DNA antibodies bound to Ars with only low affinity, and none of them was encoded by a mutated V_HId^{CR} gene. Four IgG antibodies from group III bound ssDNA; three were encoded by nonmutated V_HId^{CR} genes, and the gene that encoded the fourth had few mutations. One of the group III anti-

TABLE I
Characteristics of Hybridoma Proteins

Protein	Ars immuniza-tion	Isotype	V region gene segments					VH muta-tions	Ars binding (liters/mole)	ssDNA binding	Immuno-fluores-cence
			V_HId^{CR}	D	J_H	V_K	J_K				
Group I											
hVH65-$_{aμ}$5	0	IgM, k	+	*	4	?	?	No	0	6.7	ND
hVH65-8	0	IgM, k	+	*	4	*	4	No	0	2.7	+++
hVH65-22	0	IgM, k	+	*	3	*	?	No	0	2.7	+++‡
hVH65-25	0	IgM, k	+	*	3	*	5	No	0	2.7	+
hVH65-21	0	IgM, k	+	*	2	*	4	No	0	0.1	ND
hVH65-17	0	IgM, k	+	*	3	*	?	No	0	0.2	++
hVH65-5	0	IgM, k	+	*	1	*	1	No	0	0.1	ND
hVH65-$_{aμ}$1	0	IgM, k	+	*	3	*	1	No	0	0	0
hVH65-$_{aμ}$2	0	IgM, ?	+	*	2	?	?	No	0	0	0
hVH65-20	0	IgM, k	+	*	4	*	4	No	0	0	0
Group II											
P65D6-3	+	IgM, k	+	C	2	C	1	No	4.5×10^5	0.50	+++
P65H3-1	+	IgM, k	+	*	3	*	*	No	$<5 \times 10^4$	11.1	+
P65I4-1	+	IgM, k	+	*	2	*	*	No	$<5 \times 10^4$	1.9	+
P65D6-2	+	IgM, k	+	*	4	C	1	No	$<10^5$	0	0
P65D6-5	+	IgG2a, k	+	C	2	C	1	No	5.6×10^5	0	0
P65I4-2	+	IgG3, k	+	C	2	C	1	No	$<5 \times 10^4$	0	0
P6567-2	+	IgG1, k	+	?	4	C	1	No	$<10^5$	0	0
P65J4-1	+	IgG3, k	+	C	2	C	1	No	10^5	0	0
P65L9-3	+	IgG2a, k	+	?	1	*	*	No	$<5 \times 10^4$	0	0
P65L9-2	+	IgG1, k	+	*	1	C	1	No	4×10^5	0	0
P65C6-3	+	IgG2b, k	+	?	4	C	1	No	$<10^5$	0	0
P6567-1	+	IgG1, k	+	*	4	C	1	No	$<10^5$	0	0
Group III											
hVH65-205	++	IgG3, ?	+	*	3	?	?	No	0	0.1	+
36-65	++	IgG1, k	+	C	2	C	1	No	4×10^5	0.4	++++
44-10	++	IgG1, k	+	C	2	C	?	No	5×10^5	0.2	+
hVH65-219	++	IgG2b, k	+	*	2	C	1	Low	1.2×10^5	0.8	+
45-49	++	IgG1, k	+	*	2	C	1	High	$>10^6$	0	0
hVH65-202	++	IgG2b, k	+	*	2	C	1	No	2.5×10^5	0	0
hVH65-207	++	IgG1, k	+	C	2	C	1	Med	10^6	0	0
hVH65-208	++	IgG, k	+	C	2	C	1	High	1.6×10^6	0	ND
hVH65-209	++	IgG1, k	+	*	2	C	1	Med	9.5×10^5	0	0
hVH65-211	++	IgG3, k	+	C	2	C	1	Low	10^6	0	0
hVH65-212	;+	IgG1, k	+	C	2	C	1	Med	3.6×10^6	0	0
31-62	++	IgG2a, k	+	?	2	C	?	Low	10^6	0	++
36-71	++	IgG1, k	+	C	2	C	?	Med	4.5×10^7	0	0
16-46	++	IgG2a, k	+	?	?	?	?	Low	+	0	0
45-248	++	IgM, k	+	?	2	?	?	Low	1.7×10^6	0	ND
Group IV											
36-60	++	IgG2a, k	(C^m)	C^m	3	C^m	?	Low	1.4×10^6	0	0
36-54	++	IgM, k	(C^m)	C^m	3	C^m	?	Low	1.3×10^6	0	0
Group V											
45-112	++	IgG1, k	0	?	?	?	?	?	10^7	0	ND
hVH60-10	++	IgM, ?	0	*	3	?	?	?	0	0	0
hVH65-2	++	IgM, k	0	*	4	?	?	?	0	0	0
hVH65-7	++	IgM, k	0	*	2	?	?	?	0	0	0
$aμ3$	++	IgM, k	0	*	4	?	?	?	0	0	ND
Controls											
H130	0	IgM, k	0	*	4	?	?	?	ND	1.3	+++
TEPC 183	0	IgM, k	?	?	?	?	?	?	0	0	0
mIg	0									0	0	0

Ars immunization is designated by 0 (none), + (one injection of Ars-KLH), or ++ (two injections of Ars-KLH). All antibodies in groups I, II, and III are encoded by the V_HId^{CR} gene that partially encodes the major Id^{CR}. Antibodies in group IV are encoded by the V_H36-60 gene segment that partially encodes the minor Id 36-60. Antibodies in group V are encoded by V_H gene segments other than V_HId^{CR} or V_HId^{36-60}. Controls include H130, an anti-DNA mAb derived from MRL *lpr/lpr* mice (15); TEPC 183, a monoclonal myeloma protein that binds to neither Ars nor DNA; and pooled normal mouse immunoglobulins (mIg). V gene segments are designated as follows: C, the D or V_K region gene segments that are coexpressed with V_HId^{CR} to form the majority of anti-Ars antibodies expressed during the secondary strain A anti-Ars response; *, a D or V_K region gene segment that differs substantially from C; C^m, the V_H, D, or V_K region gene segment used to encode the minor idiotype (Id^{36-60}) of the strain A anti-Ars response. The V gene segments of H130 were identified by K. Barrett (Tufts University). The degree of somatic mutation in the V_HId^{CR} region is designated as follows: No, no evidence for somatic mutation resulting in amino acid replacements has been found by direct mRNA sequencing, NH_2-terminal amino acid sequencing, or both; Low, evidence for limited mutational alteration (≤2% amino acid substitution); Med, 2–5% amino acid substitutions; High, >5% amino acid substitution. The reaction with Ars was examined by RIA using Ars-BSA on the solid phase ([0], no detectable binding). The affinity of the reaction with Ars-tyrosine was measured by fluorescence quenching analysis, and the K_a is expressed in liters/mole. The reaction of the antibodies with DNA was studied by direct binding to ssDNA in a solid-phase ELISA. The number indicates the reciprocal (per microgram) of the amount of antibody with anti-DNA activity that was equivalent to that of 0.75 μg of the anti-DNA mAb H130. 0 indicates that 15 μg of the tested antibody had less anti-DNA activity than that of 0.75 μg of H130. The reaction with cytoskeletal structures was studied by indirect immunofluorescence (see legend to Fig. 2). 0, no immunofluorescence detected; + to ++++ indicate increasing degrees of immunofluorescence.
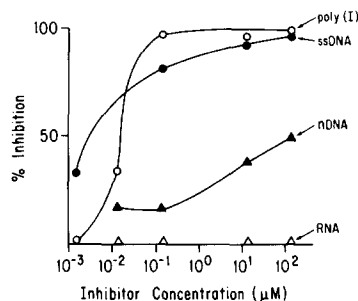
‡ An antinuclear pattern.

FIGURE 1. Inhibition of the binding of mAb hVH65-22 to ssDNA by polynucleotides. The reaction of hVH65-22 with ssDNA on the solid phase (0.45 μg/well) was examined by ELISA after preincubation of the Ig with increasing amounts of ssDNA, native DNA (nDNA), RNA, and poly(I). Poly(I) and ssDNA, but not RNA inhibited the binding of hVH65-22 to ssDNA. nDNA was a poor inhibitor. Inhibitor concentration is expressed in micromolar phosphate.

DNA antibodies, hVH65-205, had no anti-Ars activity. Another anti-DNA antibody in group III was 36-65, the prototypic Id^{CR} antibody of the anti-Ars response of A/J mice (21). Antibodies with the minor Ars idiotype (Id$^{36-60}$, group IV) and other, unrelated anti-Ars antibodies (group V) did not bind to DNA.

The specificity of the reaction with ssDNA was tested by competitive liquid-phase immunoassays (Fig. 1). ssDNA, but not RNA, a similarly charged nucleic acid, inhibited the DNA binding of all the anti-DNA antibodies. Three antibodies (hVH65-8, hVH65-22, and hVH65-25) were also tested in liquid-phase competitive assays with the synthetic polynucleotide poly(I), and with double-stranded DNA. Poly(I) completely inhibited the DNA binding of all three antibodies, whereas double-stranded DNA was only weakly inhibitory.

Immunofluorescence Analysis.   One anti-DNA mAb of group I (hVH65-22) gave a typical homogeneous antinuclear staining pattern, as well as a reaction with cytoskeletal fibers when tested with human fibroblasts (Fig. 2A). This pattern of dual nuclear and cytoplasmic reactivity has been found with mAbs derived from humans with SLE or from lupus-prone MRL *lpr/lpr* mice (3). 10 other anti-DNA antibodies also bound to cytoskeletal structures. Two antibodies bound to stress fibers (actin) in both human fibroblasts and HEP2 cells; the other eight produced immunofluorescent patterns on HEP2 substrates that were consistent with prekeratin and vimentin (Fig. 2B). When tested with human fibroblasts, those eight antibodies bound to vimentin and desmin filaments, as well as to tubulin (Fig. 2C). When colcemid-treated human fibroblasts were used as a substrate, these antibodies bound to thick perinuclear whorls, consistent with collapsed fibers of vimentin and desmin (Fig. 2D). Concordance between anti-DNA binding and immunofluorescent staining was found in all but one case, 31-62, which did not react with DNA but bound to cytoskeletal fibers (Table I).

Relationship Between Ars Binding and DNA Binding.   Six of the antibodies bound to both Ars and DNA (Table I). The affinity of their binding to Ars-tyrosine was relatively low (K_a <5 × 10^4–5 × 10^5/M). The ability of Ars-tyrosine to inhibit the reactions of these antibodies with DNA was tested (Fig. 3). Ars-tyrosine inhibited the binding of antibodies 44-10 and 36-65 (group III) to ssDNA (50% inhibition was achieved at 100 and 150 μM, respectively), but it did not inhibit the binding of P65H3-1 or P65I4-1 (group II, very low–affinity
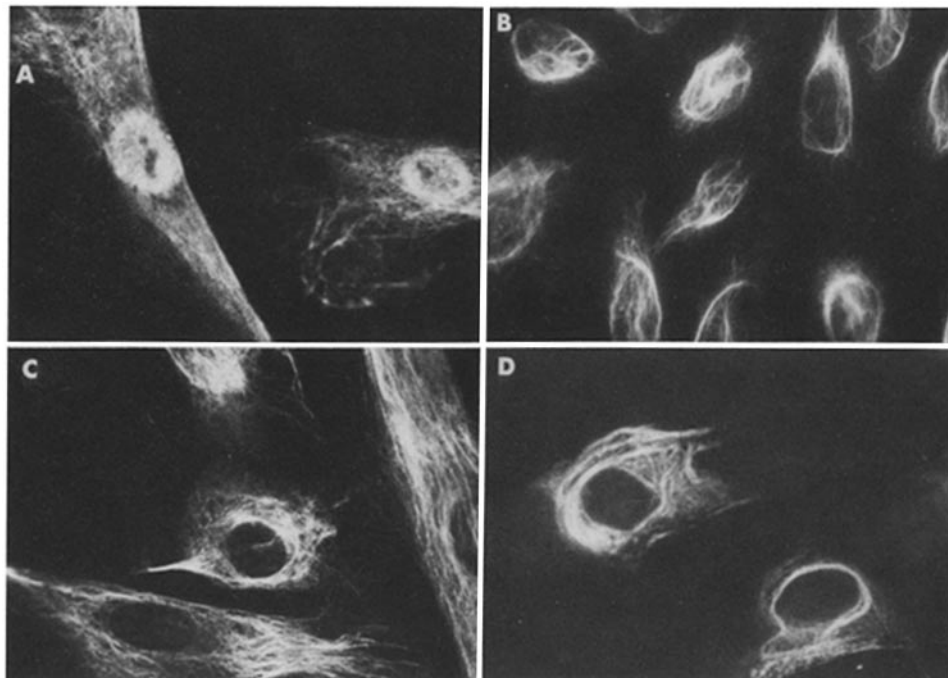
FIGURE 2. Immunofluorescent staining patterns. A, Concomitant staining of the nuclei (homogeneous pattern) and cytoplasmic fibers (vimentin) of human fibroblasts by antibody hVH65-22. B, Staining of cytoplasmic intermediate filaments (keratin and vimentin), and possibly tubulin by antibody hHv65-8 (HEP2 cells). C, Human fibroblasts with staining of desmin, vimentin fibers, and possibly tubulin by antibody hVH65-25. D, Reaction of antibody hVH65-17 with human fibroblasts treated with colcemid (5 μg/ml for 18 h). Thick perinuclear whorls characteristic of the disruption of vimentin and desmin by the drug are seen. All figures × 450.
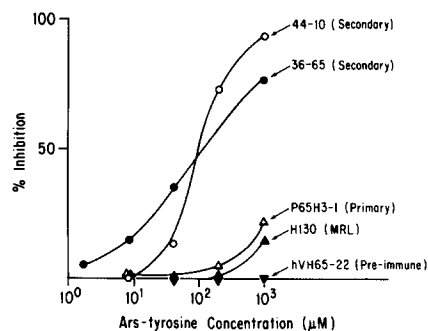

FIGURE 3. Inhibition of the binding of mAbs to DNA by Ars-tyrosine. The reaction of various anti-DNA mAbs with ssDNA on the solid phase (0.45 μg/well) was examined by ELISA after preincubation of the Igs with increasing amounts of Ars-tyrosine. The reactions of antibodies 44-10 and 35-65 with DNA, but not those of antibodies P65H3-1, hVH65-22, and H130, were inhibited by Ars-tyrosine.

anti-Ars antibodies). The hapten conjugate had no effect on the DNA binding of antibodies hVH65-8, hHV65-22, and H130, which did not bind Ars-tyrosine.

The binding of two chimeric proteins (25) to ssDNA was also tested. The Ars-binding chimera, composed of wild type heavy chain variable region sequences
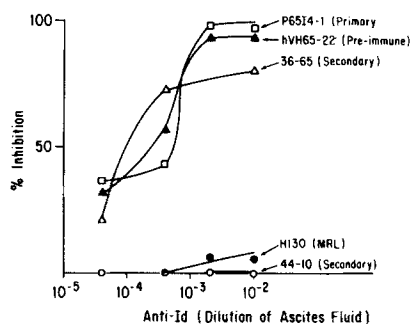
FIGURE 4. Inhibition of the binding of mAbs to DNA by antiidiotypic antibody AD8. The reaction of various mAbs with ssDNA on the solid phase was examined by ELISA after preincubation of the Igs with various dilutions of ascitic fluid of the AD8 hybridoma. The reactions of antibodies P6514-1, hVH65-22, and 36-65 with DNA, but not those of H130 and 44-10 were inhibited by the antiidiotypic antibody.

identical to those of the 36-65 antibody (a V_HC_κ chain paired with a $V_\kappa C_\kappa$ chain), also bound to ssDNA. By contrast, the chimeric protein containing a single V_H amino acid substitution (ala for ser in residue 99) that was generated by in vitro mutagenesis and has reduced Ars-binding ability (J. Sharon, manuscript in preparation), did not bind to ssDNA.

Relationship Between Id^{CR} and the DNA Binding Site. The capacity of an anti-Id^{CR} mAb (AD8) (25) to inhibit the DNA binding of six Id^{CR+} antibodies was tested. The antiidiotypic antibody inhibited the DNA binding of antibodies 36-65, hVH65-22, P6514-1, P65H3-1, and hVH65-219 (Fig. 4). The binding to DNA of antibodies 44-10 (group III) and H130 (group V), both of which lack the idiotype detected by AD8, was unaffected by the antiidiotype.

Discussion

These experiments show that an unmutated germline V_H gene segment that is expressed by preimmune B cells can encode antibodies with the typical antigen-binding properties of lupus autoantibodies. Of the 10 mAbs that had been selected by their usage of the V_HId^{CR} gene segment and obtained before immunization with Ars-KLH, 7 were found to bind to polynucleotide antigens and to cytoskeletal proteins. And of the 27 V_HId^{CR}-encoded mAbs obtained after immunization with Ars-KLH, 6 bound to DNA and cytoskeletal proteins as well as to Ars. Three of those six antibodies originated from mice that had been injected with Ars-KLH only once; they had the IgM isotype and their V_HId^{CR} genes were not mutated. The other three antibodies, derived from mice that were immunized with Ars-KLH twice, had the IgG isotype. Two of them had no mutations in their V_HId^{CR} genes and one had only a few (16, 21). One of these antibodies is 36-65, the prototypical Id^{CR+}, germline gene segment–encoded anti-Ars antibody (21). Antibody hVH65-205, which bound to DNA and cytoskeletal proteins, is also of interest. Its V_HId^{CR} gene is not mutated and, in the panel we tested, it is the only antibody obtained after immunization with Ars that does not react with the hapten (T. Manser, unpublished data). Thus, of the 14 mAbs with autoreactivity, 13 are encoded by an unmutated V_HId^{CR} gene, and in the 14th, the same gene has only a few mutations.

There are several reasons why the results are due to immunochemically specific binding of the antibodies and not to any ill-defined nonspecific sticky property of IgM. First, reactions with DNA and cytoskeletal proteins were found with both IgM and IgG antibodies; furthermore, 10 of the 20 IgM antibodies from A/J mice did not react in the autoantibody tests (Table I). The binding of the anti-DNA antibodies only to certain polynucleotides in liquid-phase assays also points to the specificity of the reactions. Both the inhibition of DNA binding by Ars-tyrosine (Fig. 3) and the loss of both ssDNA and Ars binding induced by substitution of the serine residue in position 99 of the V_H region of 36-65 indicate that the DNA-binding site of the antibodies is related to the binding site for the hapten conjugate. Finally, the inhibition of DNA binding by the monoclonal anti-Id^{CR} (AD8) (Fig. 4) adds evidence that the variable region of the mAbs was involved in the binding to DNA.

These results implicate the V_HId^{CR}-encoded heavy chain as the main contributor to the DNA-binding properties of the autoreactive antibodies in the panel we tested. However, 3 of the 13 immunoglobulins encoded by the nonmutated V_HId^{CR} gene did not bind DNA. Other portions of the antibody molecule therefore influence the expression of its potential DNA-binding site. The hybridomas were selected with a heavy chain–specific probe, and presumably expressed different light chains. Results of immunoblotting experiments with electrophoretically separated heavy and light chains from V_HId^{CR}-encoded antibodies are consistent with the interpretation that the heavy chain alone provides a DNA-binding site, and that different light chains may either permit or hinder expression of this site in the assembled molecule (Y. Naparstek and B. Ardman, unpublished results).

Even if we allow that anti-DNA antibodies are heterogeneous, and that by chance alone some V_HId^{CR}-encoded antibodies should bind DNA, the proportion of unmutated V_HId^{CR} gene–encoded antibodies that bound to DNA was high (50%). When randomly selected hybridomas from normal mice were screened for anti-DNA activity, the frequency was lower, ~10% (27). The heavy chain encoded by the unmutated V_HId^{CR} gene, therefore, appears to have a particular preference for an epitope present in DNA and cytoskeletal proteins.

The polyreactivity of anti-DNA mAbs has been attributed (28) to nonspecific or weak interactions with polyanionic molecules. However, polyreactive monoclonal autoantibodies show immunochemical specificity: only certain helical or single-stranded nucleic acid structures bind to mAb that react with multiple polynucleotides (reviewed in 2). The anti-DNA antibodies that bind to phospholipids are inhibited by diphosphatidic acid, but not by phosphatidyl serine (5), and specific polynucleotides displace the binding of polyreactive monoclonal autoantibodies to cytoskeletal proteins (3). Although ionic binding to polyanions can be shown with a variety of different proteins (29), the conditions under which we assayed anti-DNA activity (pH 7.2 or 8, ionic strength >0.1) minimize nonspecific ionic interactions. Moreover, DNA binding occurs not only with cationic Igs, but also with anionic antibodies having pIs as low as 5.3 (30). Anti-DNA antibodies that also bind to TNP do not bind to polyanions with repeating charged units like chondroitin sulfate, keratosulfate, and RNA (9). The anti-DNA antibodies we tested also failed to bind to RNA, which is just as densely

charged as ssDNA. Nor did they register in ELISA assays with blank polystyrene plates containing 3% BSA. The binding of anti-DNA antibodies may involve not only the charged surface of the polynucleotide (31), but also portions of specific bases that protrude into the major or minor grooves of the molecule (32). The latter is an important factor in determining the selectivity of the binding reactions of polyreactive anti-DNA antibodies (2). The ability of individual monoclonal anti-DNA autoantibodies to bind to other, apparently unrelated autoantigens (3–6) indicates that these apparently unrelated autoantigens must share certain epitopes. These kinds of crossreactions in preimmune antibodies suggest that autoantibodies that are generally considered to comprise distinctive, separate groups may have a common origin in the germline.

The six A/J antibodies that bound to both Ars and DNA (Table I, groups II and III) are of considerable interest. They are reminiscent of the mAbs that react with both DNA and TNP, and of the rheumatoid factors that react with both DNA and nitrophenyl hapten (8, 33–35, and reviewed in 2). This kind of dual antibody reactivity with DNA and exogenous antigens may seem strange, but as we have shown here, and as others have also demonstrated (9), the binding to DNA can be completely displaced by the synthetic hapten. The occurrence of $V_H Id^{CR}$-encoded anti-DNA antibodies within the preimmune population, and of $V_H Id^{CR}$-encoded antibodies that bind to both DNA and Ars (or only to Ars) in the immune population suggests a sequence of events in which antibodies with the dual reactivity occupy an intermediate position.

The panel of Id^{CR+} mouse mAbs tested here is analogous to the family of human antibodies that share the major crossreactive human idiotype termed $Id^{16/6}$ (36). Included in this family are autoantibodies that bind to DNA and cytoskeletal proteins, the Waldenstrom's macroglobulins that bind to capsular polysaccharides of *Klebsiella pneumoniae*, and still other macroglobulins that bind both to the autoantigens and the bacterial antigens (8). The first 40 amino-terminal residues of the light chains of four antibodies of the $Id^{16/6}$ family (16/6, 1/13b, 1/17, and 18/2), derived from different patients, are identical (37). WEA, a Waldenstrom's protein with the 16/6 idiotype, has a single substitution in that region (38), and it binds to both DNA and a *Klebsiella* polysaccharide (8). Trepicchio and Barrett[2] have pointed to the extensive structural homology of the heavy chains of antibodies of the $Id^{16/6}$ family and $V_H 26$, a human germline-encoded Ig. These structural and immunochemical findings suggest that some human autoantibodies, like the mouse antibodies described here, are part of the preimmune repertoire, and that by somatic mutation of the V genes that encode them, they acquire selective reactivity with exogenous (bacterial) antigens.

Our results support the hypothesis that autoantibodies are present in the normal preimmune repertoire and are encoded by germline genes. The data demonstrate directly that DNA-binding and anticytoskeletal activity occurs in high frequency in the preimmune germline repertoire: 13 out of 26 antibodies encoded by the unmutated $V_H Id^{CR}$ gene bound to DNA. The findings presented here are consistent with other results suggesting a high frequency of autoanti-

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[2] Trepicchio, W., Jr., and K. J. Barrett. 1986. A panel of MRL/*lpr* anti-DNA autoantibodies is encoded by multiple V_H genes derived from four V_H gene families. Manuscript submitted for publication.

bodies in the preimmune repertoire. 4–12% of hybridomas prepared from newborn (26) or germ-free (39) mice have been found to produce autoantibodies against DNA and cytoskeletal proteins, and about 6% of hybridomas from young, unimmunized mice produced antibodies reactive against pancreas, stomach, salivary gland, and pituitary (40). We conclude, therefore, that germline genes with the potential to encode autoantibodies occur in high frequency. This conclusion is consistent with Jerne's hypothesis that the available immune response repertoire is shaped by autoreactivity (41). It also appears that somatic mutation in the presence of a foreign antigen, which acts as a selective agent, can result in the loss of the autoantibody phenotype and a gain in reactivity against the foreign antigen. Recently, Mahana et al. (42) have also proposed that the antibody response to exogenous antigens originates from preimmune B cells that produce natural autoantibodies; they too found dual binding specificities (autoantigen/exogenous antigen) in mAbs derived from immunized mice. Whether the antibodies described here represent the progenitors of pathogenetic antibodies remains an open question, but since their reactions with autoantigens do not differ from those of typical lupus autoantibodies (3, 14, 15) the possibility now exists to trace the evolution of pathogenetic autoantibodies from antibodies encoded by the normal preimmune repertoire.

Summary

These experiments tested the hypothesis that unmutated germline genes from normal mice can encode autoantibodies. We found that the unmutated V_HId^{CR} gene segment, which encodes a large proportion of antiarsonate antibodies in A/J mice, also encodes antibodies with the ability to bind to DNA and cytoskeletal proteins. After Ars immunization, at a time when the V_HId^{CR} gene segment mutates and antibody affinity for the hapten increases, reactivity with the autoantigens was lost. Six antibodies obtained after immunization with Ars bound both the Ars and DNA. Results of competitive inhibition assays suggested that the same variable region site in the antibodies bound to both Ars and DNA. The properties of the individual germline–encoded antibodies, which include reactivity to both DNA and cytoskeletal proteins, suggest that autoantibodies characteristic of SLE might be a subset of antibodies encoded by unmutated germline V genes.

References

1. Christian, C. L., and K. B. Elkon. 1986. Autoantibodies to intracellular proteins. Clinical and biological significance. *Am. J. Med.* 80:53.

2. Schwartz, R. S., and B. D. Stollar. 1985. Origins of anti-DNA antibodies. *J. Clin. Invest.* 75:321.

3. André-Schwartz, J., S. K. Datta, Y. Shoenfeld, D. A. Isenberg, B. D. Stollar, and R. S. Schwartz. 1984. Binding of cytoskeletal proteins by monoclonal anti-DNA lupus autoantibodies. *Clin. Immunol. Immunopathol.* 31:261.

4. Pisetsky, D. S., S. O. Hoch, C. L. Klatt, M. A. O'Donnell, and J. D. Keene. 1985. Specificity and idiotypic analysis of a monoclonal anti-Sm antibody with anti-DNA activity. *J. Immunol.* 135:4080.

5. Lafer, E. M., J. Rauch, C. Andrzejewski, D. Mudd, B. Furie, B. Furie, R. S. Schwartz, and B. D. Stollar. 1981. Polyspecific monoclonal lupus autoantibodies reactive with both polynucleotides and phospholipids. *J. Exp. Med.* 153:897.

6. Jacob, L., F. Tron, J.-F. Bach, and D. Louvard. 1984. A monoclonal anti-DNA antibody also binds to cell-surface protein(s). *Proc. Natl. Acad. Sci. USA.* 81:3843.

7. Satoh, J., B. S. Prabhakar, M. V. Haspel, and A. L. Notkins. 1983. Human monoclonal antibodies that react with multiple endocrine organs. *N. Engl. J. Med.* 309:217.

8. Naparstek, Y., D. Duggan, A. Schattner, M. P. Madaio, F. Goni, B. Frangione, B. D. Stollar, E. A. Kabat, and R. S. Schwartz. 1985. Immunochemical similarities between monoclonal antibacterial Waldenstrom's macroglobulins and monoclonal anti-DNA lupus autoantibodies. *J. Exp. Med.* 161:1525.

9. Serban, D., C. Rordorf-Adam, Y-Z. Sun, and J. Gordon. 1985. Murine polyspecific antibodies. I. Monoclonal and serum anti-DNA antibodies crossreactive with 2,4,6-trinitrophenyl derivatives. *J. Immunol.* 135:3122.

10. Rauch, J., R. S. Schwartz, and B. D. Stollar. 1981. Applications of hybridoma technology to autoimmunity. *In* Monoclonal Antibodies and T Cell Products. D. Katz, editor. CRC Press, Inc., Boca Raton, FL. 91.

11. Diamond, B., and M. D. Scharff. 1984. Somatic mutation of the T-15 heavy chain gives rise to an antibody with autoantibody specificity. *Proc. Natl. Acad. Sci. USA.* 81:5841.

12. Kuettner, M. G., A. Wang, and A. Nisonoff. 1972. Quantitative investigation of idiotypic antibodies. VI. Idiotypic specificity as a potential genetic marker for the variable region of mouse immunoglobulin polypeptide chains. *J. Exp. Med.* 135:579.

13. Manser, T., S.-Y. Huang, and M. L. Gefter. 1984. Influence of clonal selection on the expression of immunoglobulin variable region genes. *Science (Wash. DC).* 226:1283.

14. Shoenfeld, Y., S. C. Hsu-Lin, J. E. Gabriels, L. E. Silberstein, B. C. Furie, B. Furie, B. D. Stollar, and R. S. Schwartz. 1982. Production of autoantibodies by human-human hybridomas. *J. Clin. Invest.* 70:205.

15. Andrzejewski, C., J. Rauch, E. Lafer, B. D. Stollar, and R. S. Schwartz. 1981. Antigen binding diversity and idiotypic cross-reactions among hybridoma autoantibodies to DNA. *J. Immunol.* 236:226.

16. Wysocki, L., T. Manser, and M. L. Gefter. 1986. Somatic evolution of variable region structures during an immune response. *Proc. Natl. Acad. Sci. USA.* 83:1847.

17. Kaartinen, M., G. M. Griffiths, P. H. Hamlyn, A. F. Markham, K. Karjalainen, J. L. Pelkonen, G. Makela, and C. Milstein. 1983. Anti-oxazolone hybridomas and the structure of the oxazolone idiotype. *J. Immunol.* 130:937.

18. Manser, T., and M. L. Gefter. 1984. Isolation of hybridomas expressing a specific heavy chain variable region gene segment by using a screening technique that detects mRNA sequences in whole cell lysates. *Proc. Natl. Acad. Sci. USA.* 81:2470.

19. Marshak-Rothstein, A., M. N. Margolies, J. D. Benedetto, and M. L. Gefter. 1984. Two structurally distinct and independently regulated idiotypic families associated with the A/J response to azophenylarsonate. *Eur. J. Immunol.* 11:565.

20. Margolies, M. N., A. Marshak-Rothstein, and M. L. Gefter. 1981. Structural diversity among anti-p-azophenylarsonate monoclonal antibodies from A/J mice; comparison of Id$^-$ and Id$^+$ sequences. *Mol. Immunol.* 18:1065.

21. Siekevitz, M., S.-Y. Huang, and M. L. Gefter. 1983. The genetic basis of antibody production: a single heavy chain variable region gene encodes all molecules bearing the dominant anti-arsonate idiotype in the strain A mouse. *Eur. J. Immunol.* 13:123.

22. Margolies, M. N., E. C. Juszczak, R. Near, A. Marshak-Rothstein, T. L. Rothstein,

V. L. Sato, M. Siekevitz, J. A. Smith, L. J. Wysocki, and M. L. Gefter. 1984. Structural correlates of idiotypy in the arsonate system. *Ann. N.Y. Acad. Sci.* 418:48.

23. Near, R. I., E. C. Juszczak, S.-Y. Huang, S. A. Sicari, M. N. Margolies, and M. L. Gefter. 1984. Expression and rearrangement of homologous immunoglobulin VH genes in two mouse strains. *Proc. Natl. Acad. Sci. USA.* 81:2167.

24. Rothstein, T. L., and M. L. Gefter. 1983. Affinity analysis of idiotype-positive and idiotype-negative Ars-binding hybridoma proteins and Ars-immune sera. *Mol. Immunol.* 20:161.

25. Sharon, J., M. L. Gefter, T. Manser, S. L. Morrison, V. T. Oi, and M. Ptashne. 1984. Expression of a VHC kappa chimaeric protein in mouse myeloma cells. *Nature (Lond.).* 309:364.

26. Hornbeck, P. V., and G. K. Lewis. 1983. Idiotype connectance in the immune system. I. Expression of a cross-reactive idiotype on induced anti-*p*-azophenylarsonate antibodies and on endogenous antibodies not specific for arsonate. *J. Exp. Med.* 157:1116.

27. Dighiero, G., P. Lymberi, D. Holmberg, T. Lundquist, A. Coutinho, and S. Avrameas. 1983. High frequency of natural autoantibodies in normal newborn mice. *J. Immunol.* 134:765.

28. Stott, D. I. 1985. Autoantibodies and nuclear antigens. *Immunol. Today.* 134:765.

29. Manjula, B. N., M. Potter, and C. P. J. Claudemans. 1980. The interaction of mouse myeloma immunoglobulin S15 with negatively charged polysaccharide antigens. *Mol. Immunol.* 19:913.

30. Gavalchin, J., J. A. Nicklas, J. W. Eastcott, M. P. Madaio, B. D. Stollar, R. S. Schwartz, and S. K. Datta. 1985. Lupus prone (Swr × NZB)F₁ mice produce potentially nephritogenic autoantibodies inherited from the normal SWR parent. *J. Immunol.* 134:885.

31. Ali, R., H. Dersimonian, and B. D. Stollar. 1985. Binding of monoclonal anti-native DNA autoantibodies to DNA of varying size and conformation. *Mol. Immunol.* 22:1415.

32. Ballard, D. W., and E. W. Voss, Jr. 1985. Base specificity and idiotypy of anti-DNA autoantibodies reactive with synthetic nucleic acids. *J. Immunol.* 135:3372.

33. Schubert, D., A. Jobe, and M. Cohn. 1968. Mouse myelomas producing precipitating antibodies to nucleic acid bases and/or nitrophenyl derivatives. *Nature (Lond.).* 220:882.

34. Reisen, W., and A. Morell. 1972. A human myeloma protein with specificity against dinitrophenyl and nucleic acid derivatives. *Immunochemistry.* 9:979.

35. Hannestad, K. 1969. IgM rheumatoid factors reacting with nitrophenyl groups and denatured DNA. *Ann. N.Y. Acad. Sci.* 168:63.

36. Shoenfeld, Y., D. A. Isenberg, J. Rauch, B. D. Stollar, and R. S. Schwartz. 1983. Idiotypic cross reactions of monoclonal human lupus autoantibodies. *J. Exp. Med.* 158:718.

37. Atkinson, P. M., G. W. Lampman, B. C. Furie, Y. Naparstek, R. S. Schwartz, B. D. Stollar, and B. Furie. 1985. Homology of the NH₂-terminal amino acid sequences of the heavy and light chains of human monoclonal lupus autoantibodies containing the dominant 16/6 idiotype. *J. Clin. Invest.* 75:1138.

38. Goni, F., and B. Frangione. 1983. Amino acid sequence of the Fv region of a human monoclonal IgM (protein WEA) with antibody activity against 3,4-pyruvylated galactose in Klebsiella polysaccharides K30 and K33. *Proc. Natl. Acad. Sci. USA.* 80:4832.

39. Underwood, J. R., J. S. Pedersen, P. J. Chalmers, and B. H. Toh. 1985. Hybrids from normal, germ free, nude and neonatal mice produce monoclonal autoantibodies to eight different intracellular structures. *Clin. Exp. Immunol.* 60:417.

40. Prabhakar, B. S., J. Segusa, T. Onodera, and A. L. Notkins. 1984. Lymphocytes

capable of making monoclonal autoantibodies that react with multiple organs are a common feature of the normal B cell repertoire. *J. Immunol.* 133:2815.

41. Jerne, N. K. 1974. Towards a network theory of the immune response. *Ann. Immunol. (Paris).* 125:273.
42. Guilbert, B., W. Mahana, M. Gilbert, J.-C. Mazie, and S. Avrameas. 1985. Presence of natural autoantibodies in hyperimmunized mice. *Immunology.*56:401.