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Structural and Antigenic Studies of an Idiotype-Bearing Murine Antibody to the Arsonate Hapten†

John J. Marchalonis,* Gregory W. Warr, Patricia Smith, Geoffrey S. Begg, and Francis J. Morgan

ABSTRACT: Mice of strain A/J responded to repeated intraperitoneal injection of *Limulus* hemocyanin derivatized with arsanilic acid by producing large quantities (approximately 5 mg/mL of ascites fluid) of IgG antibodies specific for this hapten. The antibodies possessed a characteristic idiotypic determinant and exhibited restricted heterogeneity as demonstrated by isoelectric focusing and primary N-terminal amino acid sequence analysis of isolated light and heavy polypeptide chains. Both light- and heavy-chain sequences were comparable to those of myeloma proteins in lack of heterogeneity. The N terminus of the light chain exhibited $V_{\kappa I}$ sequence and only one position in the first 30 residues showed more than one amino acid. No variability was observed in the first 10 N-terminal residues of the heavy chain. Rabbit antiserum to the idiotype blocked binding of hapten by the purified antibody. The presence of both light- and heavy-chain antigenic determinants was required for optimal formation of the idiotypic determinant.

It is possible, using certain mammals and immunization protocols, to raise antibodies of restricted structural heterogeneity (Appella et al., 1973; Osterland et al., 1966). Moreover, antibodies can be produced which react with the variable region combining site for antigen on the original antibodies (Capra & Kehoe, 1975). The anti-antibodies, or antiidiotypic antibodies, are useful probes for determination of the presence of antibody-like receptor molecules on the surface of lymphocytes (Binz & Wigzell, 1977; McKearn, 1974; Rajewsky & Eichmann, 1977). In this study, we induce antibodies to the arsonate hapten in strain A/J mice by a modification of the procedure of Tung & Nisonoff (1975). We report that the purified antibody was predominantly of the IgG 2a class and showed restricted heterogeneity as defined by isoelectric focusing and amino acid sequence analysis of the constituent polypeptide chains. The intact molecule exhibited idiotypic determinants, as determined by the use of rabbit antibodies produced against the $(Fab')_2$ fragment of the mouse antibody. Presence of both light- and heavy-chain determinants was required for optimal formation of the idiotypic determinant.

Materials and Methods

Antigens and Affinity Reagents. *p*-Arsanilic acid (ARS)[1] (Eastman Kodak, Rochester) was diazotized and coupled to bovine serum albumin (BSA) (crystalline, Grand Island Biological Co., New York) or *Limulus* hemocyanin (Hcyn) (prepared from hemolymph by zone electrophoresis as previously described by Marchalonis & Edelman, 1968). The arsenic content of the derivatized proteins was determined (Australian Microanalytical Service, Chemistry Department, University of Melbourne), and the molar ratios of hapten to protein were calculated as 10 mol of ARS/mol of BSA and 8 mol of ARS/Hcyn subunit of 60 000 daltons. The ARS_{10}-BSA was coupled to Sepharose 4B in a CNBr-catalyzed reaction (Haustein & Warr, 1976). The solid-phase affinity reagent contained 5 mg of derivatized BSA/mL of gel. Elution buffers consisted of pH 2.2 glycine hydrochloride (0.05 M) which was 0.15 M in NaCl and 3.5 M NaSCN (in glass-distilled H_2O).

Immunization. A/J mice maintained at the Walter and Eliza Hall Institute animal facility were immunized according to the procedure of Tung & Nisonoff (1975), with the only exception that *Limulus* Hcyn, rather than keyhole limpet Hcyn, was the carrier to which arsanilic acid was coupled. Ascites fluids were tapped after 4 weeks. A total of 24 mice were used.

Purification of Antibody to the ARS Hapten. Clarified ascites fluid was passed through the ARS_{10}-BSA immunoadsorbent. Antibody was eluted using glycine hydrochloride buffer, pH 2.2, followed by 3.5 M NaSCN.

Preparation of Polypeptide Chains. Purified antibody was reduced with 2-mercaptoethanol and alkylated with iodoacetamide, and chains were separated by gel filtration on Sephadex G-200 (Pharmacia, Uppsala) in 1 M propionic acid/4 M urea as described by Edelman & Marchalonis (1967).

Polyacrylamide Gel Electrophoresis. Polyacrylamide gel electrophoresis was performed in sodium dodecyl sulfate ($NaDodSO_4$)-containing buffers as described by Laemmli & Favre (1973).

Isoelectric Focusing. This was carried out using the LKB Multiphor flat-bed electrophoresis apparatus. Two-millimeter

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[1] Abbreviations used: ARS, *p*-arsanilic acid; BSA, bovine serum albumin; Hcyn, hemocyanin; NaCl-*P*, phosphate-buffered saline; RIA, radioimmunoassay; $NaDodSO_4$, sodium dodecyl sulfate.

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thick acrylamide gels were poured between glass plates: one was siliconized (using 2% dichlorodimethylsilane in benzene; Mallinckrodt, St. Louis, MO), and the other was acid washed. The gel solution consisted of 6.72 g of urea (ultrapure; Schwartz/Mann), 10 mL of 29.1% acrylamide (Eastman Kodak, Rochester), 10 mL of 0.9% bisacrylamide (Eastman Kodak, Rochester), 36.0 mL of water, 2.8 mL of ampholines 3.5–10, 0.4 mL of ampholines 9–11, 0.2 mL each of ampholines 6–8 and 4–6 (LKB, Rockville, MD), and 400 μL of a 0.004% solution of riboflavin (Bio-Rad, Richmond, CA). All solutions were made up using doubly glass-distilled water and were not stored longer than 1 week at 4 °C in the dark. The gel was photopolymerized, and at separation of the glass plates, it adhered to the siliconized plate. Solutions of proteins to be electrophoresed were applied (at between 1 and 3 mg/mL) to 1 × 0.5 cm pieces of Whatman No. 1 filter paper, which were placed on the gel surface. The gel was electrophoresed transversely, using wicks soaked in 1 N solutions of NaOH and H_3PO_4 at the cathode and anode, respectively. The gel was electrophoresed under conditions of controlled voltage, starting at approximately 200 V, 50 mA, and increasing to a maximum of 1000 V over a period of 1–2 h, as the current dropped to a final level of approximately 15 mA. After electrophoresis at maximum voltage for a further 2 h, the run was terminated and the pH gradient was determined directly by taking surface pH measurements, at 1 cm intervals, with a flat-surface pH electrode. The gel was then immersed in fixing solution (17.3 g of sulfosalicylic acid plus 57.5 g of trichloroacetic acid in 500 mL of water) overnight and then washed twice for 1 h in destaining solution (ethanol–acetic acid–water, 5:1.6:13.4). The gel was then stained for 20 min in 0.115% Coomassie Brilliant Blue R-250 in destaining solution at 60 °C, then destained, and finally stored in 7% acetic acid. With some care, the gel remained adherent to the glass plate throughout this procedure.

Antisera. Rabbit antisera specific for murine immunoglobulin isotypes were purchased from Litton Bionetics (Kensington, MD). Purified chicken antibody specific for the $(Fab')_2$ fragment of normal mouse IgG was prepared as previously described (Szenberg et al., 1977; Marchalonis et al., 1978; Warr et al., 1978). This reagent is specific for light-chain and Fd region determinants (Warr et al., 1978).

Preparation of Rabbit Antibodies to the Idiotypic Determinants of Mouse Anti-ARS. The $(Fab')_2$ fragment of mouse anti-ARS was prepared by cleavage with pepsin (Edelman & Marchalonis, 1967). Intact molecules (containing Fc fragments) were removed by binding to *Staphylococcus aureus* A protein coupled to Sepharose 4B (Pharmacia, Uppsala) as previously described (Szenberg et al., 1977). Two rabbits were immunized subcutaneously with 1 mg of $(Fab')_2$ in complete Freund's adjuvant. The rabbits were boosted at 3 weeks with 1 mg of antigen in incomplete Freund's adjuvant and bled 3 weeks following the booster injection. The rabbit antiserum was passed through a solid-phase immunoadsorbent of normal A/J IgG coupled to Sepharose 4B in order to remove antibodies reacting with allotypic and isotypic determinants. The IgG immunoglobulin was prepared from normal A/J serum as previously described (Szenberg et al., 1977). It was free of detectable contamination as assessed by polyacrylamide gel electrophoresis.

Amino Acid Analysis. Proteins were hydrolyzed in 6 N HCl at 110 °C for 24 h, and amino acid analysis was performed with a single narrow-bore column system (Liao et al., 1973).

NH_2-Terminal Amino Acid Sequence Analysis. NH_2-terminal amino acid sequence analysis was performed by automated phenylisothiocyanate degradation, as previously described (Edman & Begg, 1967). Each antibody chain was sequenced twice using 0.10–0.12 mol of protein. Amino acid phenylthiohydantoins were identified directly by thin-layer chromatography using solvent systems D and H and Pth-Arg and Pth-His by spot tests (Edman & Henschen, 1975). Quantitative determination of some individual Pth-amino acids was made by gas chromatography (Pisano & Brozert, 1969).

Radioimmunoassay (RIA) for Idiotypic Determinants. Four micrograms of purified antibody was iodinated, using ^{125}I as iodide (100 mCi/mL; Radiochemical Centre, Amersham), by the chloramine T method (Hunter & Greenwood, 1962) to an approximate specific activity of 25 μCi/μg. The labeled protein was separated from unbound iodide by gel filtration on a 5 × 0.5 cm column of Biogel P2 developed in phosphate-buffered saline (NaCl-*P*, 0.02 M phosphate, 0.15 M NaCl, pH 7.3). The iodinated antibody was diluted in RIA buffer (0.05 M, tris(hydroxymethyl)aminomethane, 0.15 M NaCl, 0.01% Triton X-100, 1% normal rabbit plasma) and stored at −20 °C until used. In order to determine the ability of the rabbit antiserum to recognize idiotypic determinants of the anti-ARS antibody, the following titration was carried out. Serial twofold dilutions of antiserum were made in 200 μL of RIA buffer in 10 × 75 mm round-bottom glass test tubes. To each tube was added an aliquot of diluted radioiodinated anti-ARS antibody (approximately 1 ng of protein in 25 μL of RIA buffer). The reactants were mixed thoroughly and incubated at 37 °C for 1 h and at 4 °C for 1 h. Goat antibody to rabbit IgG was then added in an amount previously determined to be capable of precipitating greater than 90% of the rabbit IgG present in the reaction mixture. After being mixed and incubated at 37 °C for 1 h and at 4 °C for 18 h, the reaction mixture was diluted with approximately 2 mL of a solution of 0.01% Triton X-100 in 0.05 M tris(hydroxymethyl)aminomethane, 0.15 M NaCl, and centrifuged at 4000*g* for 25 min at 4 °C. The supernatant was then removed from the precipitate, and the radioactivity in the precipitate was counted using a Searle well-type γ scintillation spectrometer.

The inhibition assay for specific (idiotypic) determinants on the anti-ARS antibody was carried out as follows. Serial twofold dilutions of the competing protein were made in 200 μL of RIA buffer. Rabbit antiserum to the anti-ARS antibody was added in an amount determined (in the previously described titration) to be capable of precipitating 50% of the iodinated anti-ARS antibody to be added to the mixture. After being mixed and incubated at 37 °C for 1 h and 4 °C for 1 h, the aliquot of iodinated anti-ARS antibody was added. The rest of the assay was carried out exactly as described above for the initial titration of precipitation activity. Appropriate controls were included to assess binding of iodinated anti-ARS antibody to the glass tube and precipitates of rabbit IgG/goat antirabbit IgG which did not include any rabbit antibodies to the mouse anti-ARS antibody. Results were calculated as percentage inhibition of precipitation.

Light and heavy polypeptide chains were prepared from reduced, but not alkylated, normal and idiotype-bearing IgG molecules by gel filtration in propionic acid (Edelman & Marchalonis, 1967). Intact molecules were reassembled by mixing the separated chains in propionic acid and allowing them to recombine during dialysis against 0.05 M Tris buffer, pH 7.2, 0.15 M NaCl.

Results

Demonstration and Isolation of Anti-ARS Antibodies. Ascites fluid from hyperimmunized A/J mice possessed an-

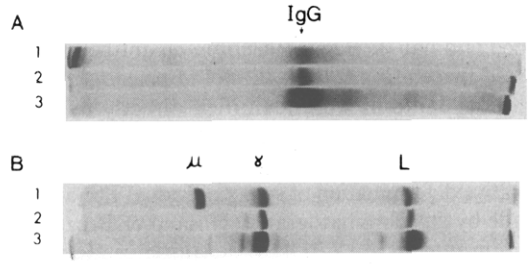

FIGURE 1: Analysis of intact (A) and reduced (B) A/J antibodies to the arsonate hapten by polyacrylamide gel electrophoresis in $NaDodSO_4$-containing buffers. A1, mixed IgM and IgG standards; A2, antibody eluted from ARS–BSA-Sepharose with 3.5 M NaSCN; A3, antibody eluted with glycine-HCl. All samples unreduced, 5% polyacrylamide. B1, IgM and IgG standard mixture; B2, antibody eluted with SCN; B3, antibody eluted with glycine hydrochloride. Samples reduced with 2-mercaptoethanol, 10% polyacrylamide.

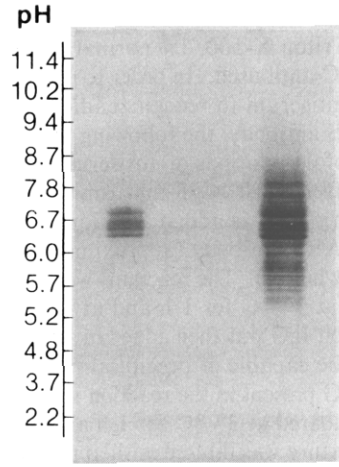

FIGURE 2: Comparison of purified A/J antibody to the arsonate hapten (left pattern) with total A/J IgG immunoglobulin (right pattern) by isoelectric focusing.

tibody to this hapten as judged by precipitation of ARS-derivatized BSA in agar gel and by quantitative precipitation of ^{125}I-labeled ARS-BSA. The antibody was purified by binding to ARS-BSA covalently coupled to Sepharose, followed by elution first with glycine hydrochloride buffer and then with 3.5 M NaSCN. In a typical experiment, 44 mg of antibody (29 with glycine hydrochloride and 15 with SCN) was isolated from 10 mL of ascites fluid. The amount of antibody isolated ranged from 4 to 6 mg/mL in various experiments. On a mass basis, the isolated antibody was 750 times more effective than the starting antibody-containing ascites fluid when tested for capacity to precipitate ^{125}I-labeled ARS-BSA.

Properties of the Anti-ARS Antibody. Figure 1 presents comparisons by polyacrylamide gel electrophoresis in $NaDodSO_4$-containing buffers of isolated anti-ARS and standard IgM and IgG immunoglobulins. The intact antibody preparations (Figure 1A) both consisted predominantly of a component comparable to IgG in gel penetration. Upon reduction (Figure 1B), the antibody preparations were resolved into components resembling light chains and γ chains in mobility. The glycine hydrochloride (A3 and B3) and SCN (A2 and B2) eluted fractions gave comparable patterns.

The isolated anti-ARS antibody migrated as a single sharp band in the γ-globulin region on the zone electrophoresis on cellulose acetate strips. This band appeared as restricted as those of homogeneous myeloma proteins. The restriction in charge heterogeneity was confirmed by isoelectric focusing (Figure 2) in which the purified antibody was compared with

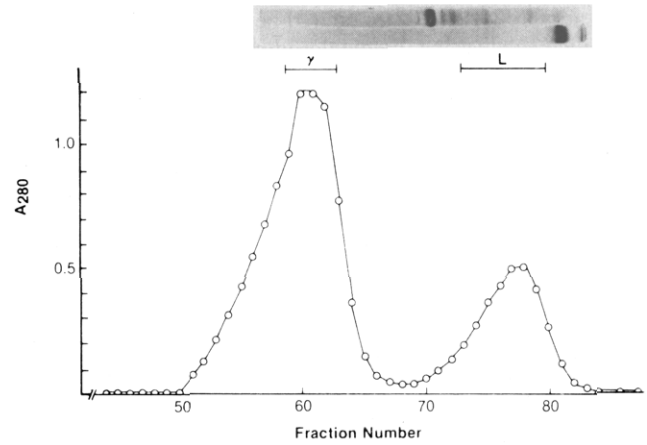

FIGURE 3: Separation of light and heavy polypeptide chains of reduced/alkylated anti-ARS antibodies by gel filtration on Sephadex G-200 in 1 M propionic acid/4 M urea. Brackets indicate fractions pooled for further analysis. The inset gives polyacrylamide gel electrophoretic patterns of the pooled γ and L fractions.

normal mouse IgG immunoglobulin. Four bands located in the pH region 6.5–7.0 were observed for the purified antibody, whereas normal IgG showed more than 20 bands ranging from pH ~5.5 to 8.7.

The purified antibody was radioiodinated and tested for its capacity to precipitate with chicken antibodies to the $(Fab')_2$ fragment of mouse IgG and with fixed *Staphylococcus aureus* bearing the A protein. As much antibody bound to protein A as was precipitated by the antiimmunoglobulin, a reagent which would precipitate all the immunoglobulin in the sample. Greater than 70% of the binding to protein A was abolished by blocking with free IgG immunoglobulin. Although the purified antibody consists only of IgG molecules, this result suggests that it contains predominantly IgG_2 and IgG_3 molecules, rather than IgG_1 which does not bind the A protein (Grey et al., 1971). This identification was confirmed using antisera directed against mouse immunoglobulin isotypes. Antiserum specific for the IgG_{2a} class exhibited clear precipitation of the antibody, whereas antibodies directed against IgG_1, IgG_{2b}, and IgG_3 gave only trace precipitations.

Polypeptide Chains of the Anti-ARS Antibody. The preceding data have shown that the anti-ARS antibody consists principally of IgG_{2a} molecules comprised of light chains and γ chains. These polypeptide chains were resolved in milligram quantities from reduced and completely alkylated IgG by gel filtration on Sephadex G-200 in 1 M propionic acid/4 M urea (Figure 3). As shown in the inset, light chains were obtained free of contamination by γ chains. The γ-chain preparation showed trace contamination by bands of mobility intermediate between light and γ chains. Since these contaminants were not present in the starting material (Figure 1), it is presumed that they represent breakdown products of the γ chain.

Amino Acid Composition and Partial Sequence of the Anti-ARS Polypeptide Chains. The amino acid composition of the anti-ARS light chain is given in Table I. These values are typical of those of mouse κ chains (Svasti & Milstein, 1972). NH_2-terminal amino acid sequence analysis was carried out as described by Edman & Begg (1967), and 30 residues of light chain (Table II) and 10 residues of γ chain (Table III) were determined. The repetitive yield was 95–96%. Overlap, estimated as less than 5%, was essentially undetectable by thin-layer chromatography, and unambiguous identification of all residues was made by qualitative thin-layer chromatography except in the one instance described below. Both chains were homogeneous as judged from the results of

Table I: Amino Acid Analysis of the Light Chain of A/J Antibody to the Arsonate Hapten[a]

amino acid	residues/ 100 residues	amino acid	residues/ 100 residues
Asp	12.0	Met	1.1
Thr	10.0	Ile	4.6
Ser	13.9	Leu	7.4
Glu	10.5	Tyr	4.5
Pro	3.8	Phe	3.5
Gly	7.2	His	1.5
Ala	4.3	Lys	5.7
$^1/_2$-Cys	1.5[b]	Arg	4.0
Val	4.3	Trp	c

[a] Hydrolysis for 24 h; no corrections have been made. [b] Determined as half-cystine. [c] Not determined.

the initial cycles of the Edman degradation. One sequence only was obtained for the γ chain. The light chain gave a single sequence except at position 3 where Val and Gln were present in the ratio of approximately 1:2 as determined by gas chromatography. At step 8 the amount of Pth-Thr observed was somewhat higher than could readily be accounted for by overlap from step 7, and the possibility that some light-chain molecules have threonine at step 8 cannot be excluded at this stage; this point should be resolved during further sequence studies. A gap was seen, at step 23 where Cys was assumed by homology with κ chains, as Pth-Cys is unstable in the conversion reaction. The first 23 residues of the light chain are extremely similar to those of a κ myeloma protein (MOPC 41) typical of the $V_{\kappa I}$ subgroup (Hood et al., 1970). The anti-ARS heavy chain showed marked similarity to all of the

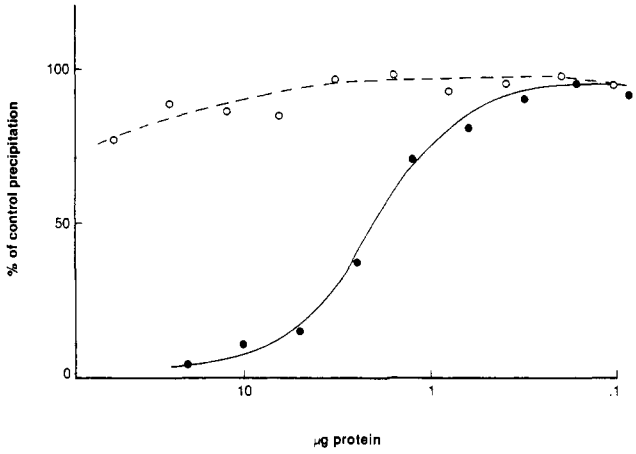

FIGURE 4: Competition radioimmunoassay to detect idiotypic determinants on purified A/J antibody to the arsonate hapten (anti-ARS). Inhibition of precipitation of ^{125}I-labeled anti-ARS antibody by the adsorbed rabbit antiserum to the $(Fab')_2$ fragments of the anti-ARS antibody is shown for normal A/J IgG (○) or the anti-ARS antibody (●).

V_H prototype sequences but differed least from the V_{HII} sequence (80% identity).

An Idiotypic Determinant on the Anti-ARS Molecule. Rabbit antiserum to the $(Fab')_2$ fragment of the anti-ARS molecule was rendered specific for the individual determinants of this molecule by solid-phase absorption with the normal A/J IgG fraction from ascites fluid which did not bind ARS. That the absorbed rabbit antiserum recognized characteristic anti-ARS determinants is shown by the competition RIA data

Table II: N-Terminal Sequences of Light Chains of Antiarsonate Antibodies and of a Murine κ-Chain Myeloma Protein

	residue number														
light chain	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A/J anti-ARS[a]	Asp	Ile	Val Gln	Met	Thr	Gln	Thr	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Leu
A/J anti-ARS[b]	Asp	Ile	Gln Val	Met	Thr	Gln	Thr	Pro	Ser	Ser Ile	Leu	Ser Leu	Ala	Ser	Leu
rabbit[c] anti-ARS	Ala Asp	Asp Val Glu	Ile Val	Val Met	Met Thr	Thr Gln	Gln Thr Pro	Thr Pro	Pro Ala	Ala Ser					
MOPC 41[d] ($V_{\kappa I}$)	Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Leu

	residue number														
	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
A/J anti-ARS[a]	Gly	Asp	Arg	Val	Ser	Ile	Ser	(Cys)	Arg	Ala	Ser	Gln	Asx	Ile	Ser
A/J anti-ARS[b]	Gly	Asp	Arg	Val	Ser	Ile	Ser Leu	Cys	Arg	Ala	Ser	Gln	Asp	Leu	Ser
MOPC 41[d] ($V_{\kappa I}$)	Gly	Glu	Arg	Val	Ser	Leu	Thr	Cys	Arg	Ala	Ser	Gln	Asx	Ile	Gly

[a] Present data. [b] Data of Capra et al., 1975a, 1977. [c] Data of Fraser & Edman, 1970. [d] Data of Gray et al., 1967.

Table III: N-Terminal Sequences of Heavy Chains of Purified A/J Antibody to the Arsonate Hapten. Comparison with Murine V_H Subgroups

	residue number									
heavy chain	1	2	3	4	5	6	7	8	9	10
A/J anti-ARS[a]	Glu	Val	Gln	Leu	Gln	Gln	Ser	Gly	Ala	Glu
V_{HI} (MOPC 460)[b]	Glu	Val	Gln	Leu	Gln	Glu	Ser	Gly	Pro	Ser
V_{HII} (MOPC 104E)[b]	Glu	Val	Gln	Leu	Gln	Glu	Ser	Gly	Pro	Glu
V_{HIII} (MOPC 173)[c]	Glu	Val	Lys	Leu	Leu	Glu	Ser	Gly	Gly	Pro

[a] Present data. [b] Data of Barstad et al., 1974. [c] Data of Fougereau et al., 1976.

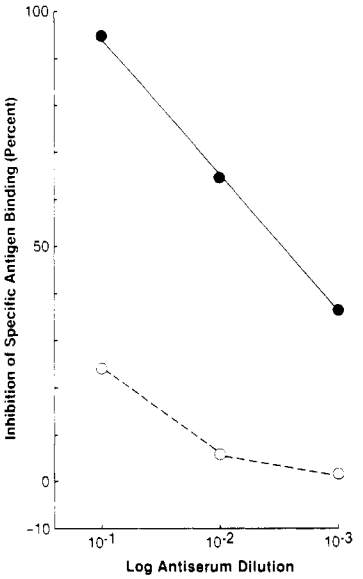

FIGURE 5: Blocking by rabbit antibody to the idiotype of A/J anti-ARS antibody of binding of ^{125}I-labeled anti-ARS to ARS-BSA-Sepharose (●). This antibody does not specifically block binding of ^{125}I-labeled MOPC 315 IgA myeloma protein to DNP-BSA-Sepharose (○).

of Figure 4. Two micrograms of anti-ARS gives 50% inhibition of specific precipitation of this molecule, whereas 50 μg of normal mouse IgG is ineffective in the competition. The absorbed anti-ARS antibody is directed toward combining-site determinants as illustrated in Figure 5. The anti-ARS selectively inhibits binding of ^{125}I-labeled anti-ARS to ARS-BSA-Sepharose, compared to its effect on binding of ^{125}I-labeled MOPC 315 IgA to its specific ligand dinitrophenol on dinitrophenol-BSA-Sepharose. Rabbit antibodies to normal mouse κ chain gave less than 30% inhibition at a 10^{-1} dilution.

Experiments with isolated polypeptide chains and recombinant molecules showed that the specific light and γ chain were both required for efficient formation of the idiotypic determinant (Table IV). However, recombinant molecules formed of anti-ARS γ and normal light chains showed inhibition better than that of anti-ARS light/normal γ recombinant molecules, suggesting that the γ and L chains may not contribute equally to this determinant. The anti-ARS γ chain exhibited substantial binding capacity when combined with pooled light chain, but combination with the specific light chain was required for full recovery of the idiotypic determinant. Some reconstitution of activity was observed when specific light or heavy chains were combined with normal heavy or light chains, indicating that chain–chain interaction is required to form the determinant.

Discussion

We have performed detailed serological and biochemical studies of antibodies to ARS produced by strain A/J mice in response to repeated intraperitoneal injection of ARS-derivatized *Limulus* Hcyn. These antibodies consisted predominantly of IgG_{2a} molecules characterized by restricted heterogeneity and bearing a characteristic idiotype. The N-terminal V-region sequences of the light (30 residues) and heavy chain (10 residues) were as homogeneous as those obtained in sequencing a myeloma protein. Our results with strain A/J antibodies to ARS differ from those of Tung & Nisonoff (1975) inasmuch as their cross-reactive anti-ARS antibodies comprised a family of IgG_1 molecules, and the light chains showed considerable sequence heterogeneity (Capra et al., 1975a). However, our N-terminal sequences are virtually

Table IV: Inhibition of Precipitation of Anti-ARS Antibody by Antiidiotypic Reagents

preparation	amt of antibody giving 50% inhibn of precipitation (pM)
normal MIgG	NSI[a]
normal MIgG γ chains	NSI
normal MIgG L chains	NSI
idiotype-bearing anti-ARS antibody	2.8
idiotype anti-ARS γ chain	18.0
idiotype anti-ARS L chain	150.0
normal γ/normal L recombinant	NSI
normal γ/anti-ARS L recombinant	50.0
anti-ARS γ/normal L recombinant	7.0
anti-ARS γ/anti-ARS L recombinant	2.0

[a] NSI = no significant inhibition.

identical with the dominant sequences reported by those workers (Capra et al., 1977). The only difference in the first 30 N-terminal light-chain residues was that we found Ile at position 29 while Capra et al. (1977) report Leu (see Table II). The first N-terminal 10 residues of the γ chain are identical with those of Capra et al. (1975b). Thus, it appears that V-region framework residues of the two antibodies are very similar and it is now necessary to determine whether or not the same specificity-determining hypervariable regions are shared by both. This question is of interest because of the difference in isotypes between the two antibodies, although distinct isotypes have previously been shown to share V_H regions (Gearhart et al., 1975), and the fact that the A/J mice used in this study had been bred in Australia for several generations. If the generation of hypervariable-region diversity occurs on an episome distinct from the V-region structural genes (Capra & Kehoe, 1974), it is possible that particular framework sequences are not associated with hypervariable sequences in an obligatory manner. Further comparisons between the V-region sequences of the anti-ARS chains described here and those of Capra et al. (1977) should bear upon this question.

An interspecies comparison of the present light-chain data with the first 10 N-terminal residues of light chains of purified rabbit antibodies to the ARS hapten (Fraser & Edman, 1970) shows 80% identity in sequence (see Table II). This result does not bear upon the hypervariable-region residues which regulate the specificity for hapten, but it suggests that the light chains probably represent the same κ subgroup. The rabbit antibody showed considerable heterogeneity with respect to both the present results with A/J antibodies to ARS and those of Capra et al. (1975a, 1977).

The present data show that formation of the complete idiotypic determinant strictly requires the presence of both light and heavy chains of the anti-ARS molecule. This result is important to studies of the molecular nature of antigen receptors on thymus-derived lymphocytes because Goodman et al. (1978) have provided preliminary evidence for the cross-reactive anti-ARS idiotype on T cells specific for the ARS hapten. Although it is now accepted that immunoglobulin idiotypes occur on specific T cells, most recent reports indicate that these consist solely or predominantly of heavy-chain determinants (Binz & Wigzell, 1977; Rajewsky & Eichmann, 1977). A recent genetic study by Laskin et al. (1977), however, indicates that loci governing light-chain expression may contribute to inheritance of the anti-Ar (ARS) idiotype. This result is consistent with the recombination data

presented here which parallel classic studies showing that optimal reconstitution of antibody activity by assembling antibodies from isolated light and heavy chains requires that both heavy and light chains of the proper specificity are present in the recombinant molecule (Fougereau et al., 1964). Definitive proof of the existence of the anti-ARS idiotype on T cells would show that the controversial T cell receptor occurs in a form directly similar to that of classical immunoglobulins in its combining site, although its isotypic markers are apparently distinct from those of classical immunoglobulin (Cone, 1977; Marchalonis, 1977; Moseley et al., 1977). Studies are in progress to delineate the cellular distribution of this idiotype. Preliminary observations from this laboratory (G. W. Warr, D. DeLuca, and J. J. Marchalonis, unpublished results) show that a few percent of peripheral T cells of A/J mice immunized with ARS-Hcyn bear the anti-ARS idiotype described here, a situation similar to that reported by Goodman et al. (1978).

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