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Amino Acid Sequence of the Heavy Chain Variable Region from the A/J Mouse Anti-Arsonate Monoclonal Antibody 36-60 Bearing a Minor Idiotype†

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ABSTRACT: In addition to the predominant idiotype family (Id^{CR}) associated with the murine A/J anti-azophenylarsonate response, a second idiotype family (Id^{36-60}) was defined on the basis of serologic cross-reactivity and amino acid sequence homology among monoclonal antibodies [Marshak-Rothstein, A., Margolies, M. N., Benedetto, J. D., & Gefter, M. L. (1981) *Eur. J. Immunol. 11*, 565–572]. The complete variable region amino acid sequence of the A/J IgG2a hybridoma protein 36-60 heavy chain was obtained by automated Edman degradation of the intact chain and fragments generated by cleavage with CNBr and by tryptic digestion of a succinylated CNBr peptide. A comparison of the Id^{36-60} heavy chain sequence to that of the Id^{CR} heavy chain variable region reveals only 45% homology between them. The structural data indicate that different genes encode the V_H, D, and J_H gene-encoded sequences in the two idiotypes.

Idiotypes are serologically defined markers on immunoglobulin variable regions that often involve the antigen combining site. Idiotypes in inbred strains of mice have been instrumental in the understanding of antibody diversity and immune regulation. Certain predominant idiotypes may be present on the majority of antibodies synthesized in selected immune responses. The major cross-reacting idiotype (here designated Id^{CR})[1] in A/J mice immunized with *p*-azophenylarsonate (Ars) linked to protein carriers is an example of such a response (Kuettner et al., 1972). The origin of diversity in this Ars-associated idiotype has been addressed through structural studies both at the DNA level (Siekevitz et al., 1982, 1983; Sims et al., 1982) and of the protein products (Estess et al., 1979, 1980; Marshak-Rothstein et al., 1980; Alkan et al., 1980; Margolies et al., 1981, 1983; Siegelman & Capra, 1981). The somatic cell fusion technique has made it possible to structurally characterize monoclonal antibodies of the desired idiotype. In the course of an examination of several randomly selected hybridoma proteins lacking the predominant Id^{CR}, a second idiotype family (Id^{36-60}) was defined, which also consists of a set of closely related molecules. Hybridoma proteins bearing Id^{36-60} were regulated independently of Id^{CR} in suppression experiments (Marshak-Rothstein et al., 1981). The frequent appearance of both Id^{CR} and Id^{36-60} among anti-Ars antibodies suggested that they each arise directly from germ-line genes. As it is not known why Id^{CR} predominates in A/J mice, it is important to establish the degree of phenotypic diversity in these two families of monoclonal antibodies and to relate this diversity to the number and sequence of the relevant germ-line genes.

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[1] Abbreviations: Id^{CR}, a predominant cross-reacting idiotype in the A/J strain of mouse defined by rabbit antisera; Ars, *p*-azophenylarsonate; Id^{36-60}, a second idiotype expressed by a subpopulation of anti-Ars antibodies in A/J mice; HPLC, high-pressure liquid chromatography; Pth, phenylthiohydantoin; KLH, keyhole limpet hemocyanin; CDR, complementarity-determining region; BSA, bovine serum albumin; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; Gdn·HCl, guanidine hydrochloride; SDS, sodium dodecyl sulfate.

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We report here the complete heavy chain variable region amino acid sequence of the anti-Ars hybridoma protein 36-60. The 36-60 heavy chain V-region sequence is derived from the 36-60 V_H germ-line gene sequence (R. Near and M. Gefter, unpublished results) by only a few somatic mutations. The sequence differs markedly in the V_H, D, and J_H gene-encoded segments from that of Id^{CR} heavy chains.

Materials and Methods

Hybridoma Cell Lines. The derivation of the cell line 36-60 secreting an anti-Ars hybridoma protein has been described in detail previously (Marshak-Rothstein et al., 1980). Briefly, spleen cells from A/J mice immunized with Ars–KLH conjugates were fused with cells of the nonsecreting BALB/c myeloma cell line Sp2/0-Ag14. The IgG2a hybridoma 36-60 lacks the predominant cross-reacting idiotype Id^{CR} (Marshak-Rothstein et al., 1980) but was used to define a minor idiotype (Id^{36-60}) structurally distinct from Id^{CR} (Marshak-Rothstein et al., 1981). Hybridoma protein 36-60 was amplified in ascites in (A/J × BALB/c) F1 mice. The mice were obtained from Jackson Laboratories (Bar Harbor, ME).

Purification of Hybridoma Protein. Hybridoma protein 36-60 was purified by affinity chromatography on columns of Ars–BSA–Sepharose 4B as described previously (Margolies et al., 1981). Fractions obtained by affinity chromatography were screened for the presence of antibody by electrophoresis on cellulose acetate strips. The yield of purified antibody was 2–3 mg/mL of ascites.

Preparation of Heavy Chains. Antibodies eluted from affinity columns with 3 M KSCN were dialyzed against 0.15 M NaCl–0.01 M K_2HPO_4 buffer, pH 7.4. Following concentration by vacuum dialysis or lyophilization, antibody was partially reduced in 0.5 M Tris-HCl (pH 8.2) containing 5 mM EDTA and alkylated with iodoacetic acid, as described (Novotny & Margolies, 1983). Separation of heavy and light chains was carried out on columns of AcA 44 (LKB), as described (Novotny & Margolies, 1983).

Complete Reduction and Alkylation. Complete reduction and alkylation were performed as described previously (Novotny & Margolies, 1983) except that 25 μCi of iodo[1-^{14}C]acetic acid (New England Nuclear) was incorporated during alkylation.

CNBr Cleavage. Partially reduced and alkylated heavy chains (2 μmol) were dissolved in 2–3 mL of 70% formic acid. Two hundred milligrams of CNBr (Eastman) was added, and cleavage proceeded for 24 h at room temperature. The mixture was degassed at low vacuum and applied to a Sephadex G-100 column (2.5 × 200 cm) in 20% formic acid. The column effluent was monitored by the absorbance at 280 nm. Fractions of 4 mL were collected.

Modification of Lysine Residues and Trypsin Digestion. Heavy chain variable region CNBr peptides were dissolved in 2 mL of 0.2 M sodium borate buffer, pH 9.0, containing 7 M guanidine hydrochloride. Solid succinic anhydride (Sigma) was added in portions until at least a 10-fold molar excess relative to lysine residues was obtained. The pH was maintained at 9.0 by the addition of 2 M NaOH. The sample was then dialyzed in Spectrapor D dialysis tubing overnight against 1% ammonium bicarbonate (pH 8.0). Trypsin digestion of modified peptides was performed as described previously (Cannon et al., 1978). The lyophilized trypsin digests were dissolved in a minimal amount (2 mL) of 0.1 M sodium acetate–5 M guanidine hydrochloride buffer, pH 5.5, and subjected to gel filtration on a Sephadex G-75 column (2.0 × 120 cm) in the same buffer. Fractions of 4 mL were collected. Pooled fractions were freed from salt by dialysis in Spectrapor H dialysis tubing. The smallest peptides were desalted on columns of Sephadex G-10 (2.5 × 50 cm) in 0.03 M NH_4OH and lyophilized.

Amino Acid Analysis. Amino acid compositions were determined by using a Dionex D-500 analyzer following hydrolysis of samples in sealed evacuated tubes at 110 °C for 24 h in constant-boiling HCl.

Amino Acid Sequence Methods. Automated Edman degradation was performed in a Beckman Model 890-C sequencer equipped with a Beckman cold trap and a Sequemat Model SC-150 sequential controller (Sequemat, Inc., Watertown, MA). A 0.1 M Quadrol program (Brauer et al., 1975) which employs a single acid cleavage (3 min) was used. At cycles N terminal in proline, the program was altered to include two successive acid cleavages. Polybrene (Tarr et al., 1978; Klapper et al., 1978) was added to the sequencing cup prior to degradation of peptides. A Sequemat Model P-6 autoconverter was used for the conversion of anilinothiazolinone-amino acids to the Pth derivatives by using methanol–HCl (Margolies et al., 1982). All of the Pth-amino acids, as well as the Pth derivatives of *S*-(carboxymethyl)cysteine and ε-succinyllysine, were identified by high-pressure liquid chromatography on Zorbax ODS columns (Du Pont) as previously described (Margolies & Brauer, 1978; Margolies et al., 1982). At cycles where proline was N terminal, *o*-phthaldehyde was added to the cup in butyl chloride from a separate reagent bottle (R_4), based on the method described by Bhown et al. (1981). Each residue was identified in at least two sequencer degradations.

Results

Partially reduced and alkylated heavy chain (30 nmol) was subjected to automated Edman degradation. As a proline residue was identified in the initial degradation at cycle 41, *o*-phthaldehyde treatment was used in a subsequent degradation at this cycle prior to coupling, followed by two successive acid cleavages (6 min). This treatment reduced the background sufficiently to permit positive identification of Pth-amino acids for 60 successive cycles except for tyrosine-59 on the intact heavy chain (Figure 4). The repetitive yield was 94%, and the initial yield was 32 nmol. A methionine residue was identified at cycle 48; therefore, CNBr cleavage was used to generate an overlap sequence.

Partially reduced and alkylated heavy chains (2 μmol) were subjected to cleavage with CNBr. Separation of the CNBr peptides was initially attempted on AcA 44 columns in 5 M Gdn·HCl–0.1 M sodium acetate buffer (pH 5.5), but aggregation of V-region peptides interfered with adequate purification. Thereafter, CNBr peptides were separated on Sephadex G-100 columns in 20% formic acid (Figure 1). The peptide contained in pool I was lyophilized. An aliquot (10 nmol) was subjected to Edman degradation, revealing two Pth-amino acids at each cycle, consistent with sequences beginning at the amino terminus of the heavy chain and also at position 49 (Figure 4). As the first constant-region methionine in IgG2a (Fougereau et al., 1976) is located at position 265 [numbering according to Kabat et al. (1979)], the sequence results are consistent with the presence of two peptides in pool I (1–48 and 49–265) linked by a disulfide bridge, accounting for the entire variable region. The other CNBr peptides presumably originating from the constant region (Figure 1) were therefore not examined further. The two peptides contained in pool I (Figure 1) were separated by gel filtration on Sephadex G-75 (Figure 2), following complete reduction and alkylation. The peptide in pool II (Figure 2) was dialyzed extensively against water in Spectrapor H tubing and lyo-

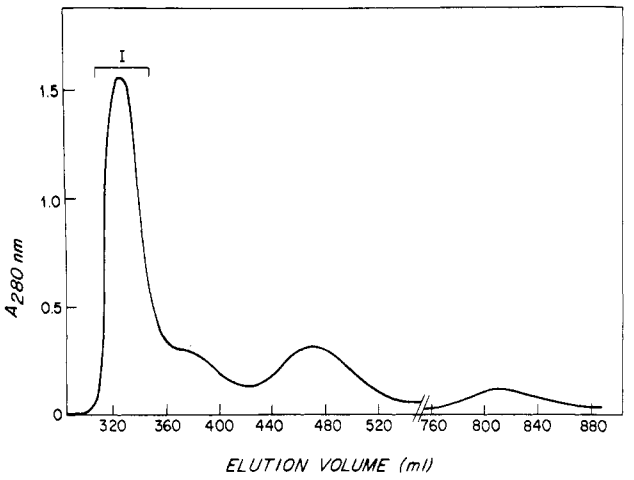

FIGURE 1: Fractionation of CNBr peptides of the 36-60 heavy chain (900 nmol) on a column of Sephadex G-100 (2.5 × 200 cm). The column was equilibrated in 20% formic acid. Fractions of 4 mL were collected. Fractions contained within the bracket were pooled and lyophilized.

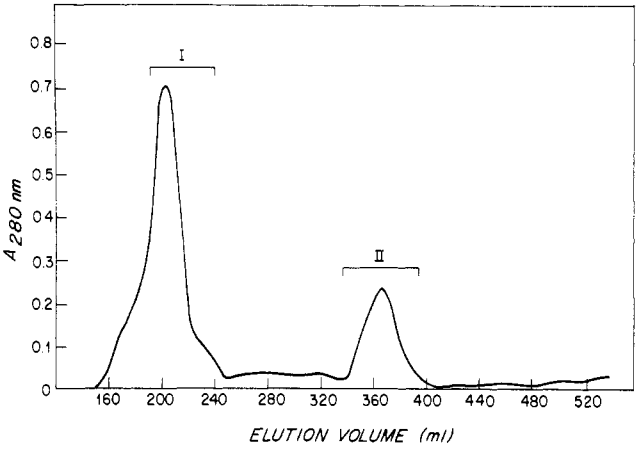

FIGURE 2: Fractionation of variable-region CNBr peptides on a Sephadex G-75 column (2.5 × 120 cm) in 5 M Gdn·HCl–0.1 M sodium acetate buffer, pH 5.5 The CNBr peptides contained in pool I (Figure 1) from partially reduced and alkylated heavy chains were subjected to complete reduction and alkylation to cleave the disulfide bridge between peptide CB-1 (pool II, residues 1–48) and peptide CB-2 (pool I, residues 49–265). Fractions (4 mL) contained within the brackets were pooled, dialyzed against water, and lyophilized.

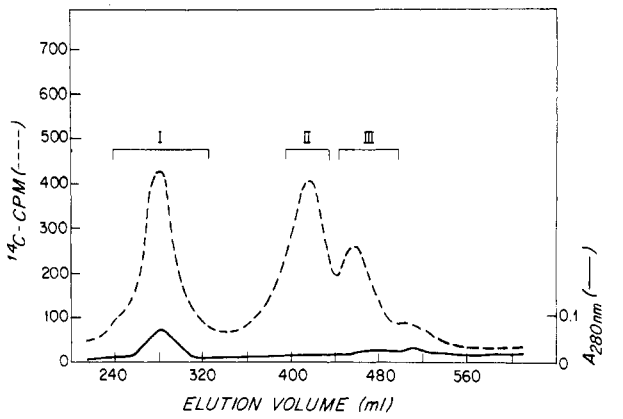

FIGURE 3: Gel filtration of a tryptic digest of completely reduced and alkylated succinylated peptide CB-2. The digest (800 nmol) was applied to a column of Sephadex G-75 (2.5 × 120 cm) equilibrated in 5 M Gdn·HCl–0.1 M sodium acetate buffer, pH 5.5. Fractions (4 mL) were pooled as indicated by the brackets. Pools I and II were dialyzed in Spectrapor D tubing against water and lyophilized. The peptide in pool III was freed from salt on a column of Sephadex G-10 (2.5 × 50 cm) in 0.03 M NH_4OH and lyophilized. Pool I contained fragment R-2 (residues 100–228), and pool III contained fragment R-1 (residues 72–99; see Figure 4). Pool II was assumed to contain a constant-region peptide including residues 229–265.

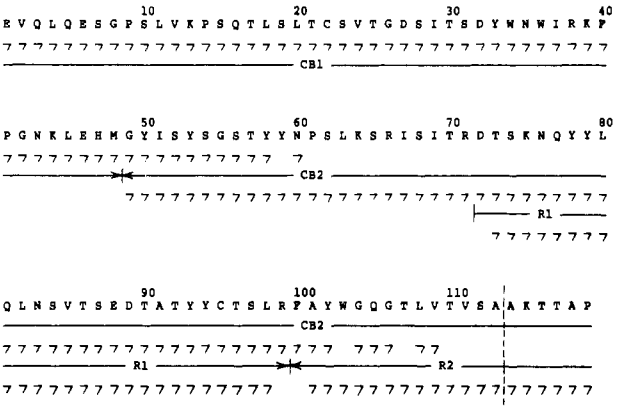

FIGURE 4: Variable region amino acid sequence of the anti-Ars hybridoma heavy chain 36-60 bearing a minor idiotype. The major peptides used to establish the sequence are indicated: CB and R are used to denote peptides obtained following cleavage by CNBr and by trypsin (following succinylation), respectively. Results of Edman degradation on the intact heavy chain are indicated by arrows below the printed sequence. Results obtained by the sequence of individual fragments are indicated by arrows below the designated fragments. A simple consecutive numbering system is used. (The constant-region CH_1 domain begins at residue 114.) A one-letter code for amino acid residues is used (IUPAC-IUB Commission on Biochemical Nomenclature, 1968).

philized. Sequence analysis of an aliquot (5 nmol) of pool II revealed the presence of peptide CB-1 (residues 1–48) and a contaminating constant-region peptide (residues 438–477). Pool I was dialyzed extensively against water and lyophilized. In an initial automated sequence analysis (15 nmol), residues 49–80 of the variable region were identified (Figure 4, peptide CB-2), but contamination due to peptide CB-1 was present. In subsequent preparations of pool I, peptide CB-2 was successfully degraded further. In this instance, contaminating peptides were not observed. Automated Edman degradation of 40 nmol of the peptide in the presence of Polybrene permitted positive identification of 57 out of 59 cycles (Figure 4). The average repetitive yield was 96.6%. Treatment with *o*-phthaldehyde was used at cycle 13 (position 61, Figure 4). These results proved the presence of an arginine-phenylalanine sequence at positions 99-100, confirming that there were no intervening peptides between peptides R-1 and R-2 (discussed below) (Figure 4).

The presence of an arginine residue at position 71 in the variable region, identified during degradation of peptide CB-2 (Figure 4), prompted the generation of tryptic peptides from peptide CB-2. Peptide CB-2 (800 nmol) was succinylated and digested with trypsin. Resolution of these arginine peptides by gel filtration on Sephadex G-75 is illustrated in Figure 3. Pool I was dialyzed extensively against water and lyophilized. An aliquot (40 nmol) revealed an amino acid sequence consistent with a peptide with an N-terminal residue at position 100 (Figure 4). This peptide (R-2) was sequenced for 27 cycles (repetitive yield 92%). The sequence of peptide R-2 following cycle 14 (positions 114–126 in Figure 4) was identical with the known CH_1-domain sequence for the IgG2a isotype (Fougereau et al., 1976), thus identifying peptide R-2 as the C-terminal arginine peptide of the variable region. A single sequence was found except at cycle 1, where the identification of Pth-phenylalanine was tentative because of the presence of contaminating Pth-amino acids of glycine and alanine. Pth-phenylalanine was, however, identified as the only residue at this position in a degradation on peptide CB-2

(residues 49–265). The peptide contained in pool III was freed from salt on a column of Sephadex G-10. Sequence analysis (15 nmol) revealed a single sequence for 24 cycles (repetitive yield 91%). The amino acid sequence found was consistent with the peptide beginning at position 72, based on residues which overlap those of peptide CB-2 (Figure 4). Cycle 1 (residue 72) was not identified but was known to be aspartic acid from previous analyses of the parent peptide CB-2. In addition, a C-terminal arginine residue (later shown to be present at position 96; Figure 4) was not identified.

As the known IgG2a constant-region sequence of peptide CB-2 (49–265) contains a single arginine residue at position 228, pool II was assumed to contain the C-region peptide extending from position 229 to 265 and was not examined further. The sequence analyses of peptides R-1 (positions 72–99) and R-2 (positions 100–126), together with the sequence analysis of peptide CB-2 (positions 49–265), which provides the overlap between R-1 and R-2, completed the sequence of the variable region (Figure 4).

Discussion

The 36-60 hybridoma protein was homogeneous following affinity purification as judged by SDS gel electrophoresis and extended amino-terminal sequence analysis of both heavy and light chains (Marshak-Rothstein et al., 1981). The production of hybridoma proteins secreted in ascites resulted in ample amounts of protein for sequence analysis (2–3 mg of purified antibody/mL of ascites). Following initial Edman degradation of this intact IgG2a heavy chain, the strategy for cleavage involved production of large CNBr fragments (residues 1–48 and 49–265, linked by a disulfide bridge). These fragments contained the entire variable region and the CH_1 domain. The variable-region sequence was completed by extended Edman degradation of the larger CNBr fragment (peptide CB-2, residues 49–265) and by sequence analysis of tryptic peptides from the succinylated CB-2 peptide. These sequence analyses provided overlaps spanning all the fragments, a necessity in light of the unusually short third complementarity-determining region (CDR) (Figure 4). The length of useful Edman degradation was enhanced by the use of *o*-phthaldehyde to eliminate background and overlap at cycles where proline is amino terminal in the remaining peptide. The amino acid sequence of the 36-60 heavy chain variable region is in complete agreement with the sequence of rearranged DNA from the 36-60 hybridoma, determined independently by R. Near and M. Gefter (unpublished results).

Nisonoff and co-workers described and characterized a heritable idiotype represented in 20–70% of anti-Ars antibodies in all strain A mice immunized with Ars–protein conjugates (Kuettner et al., 1972). The appearance of this idiotype (here denoted Id^{CR}) could be suppressed by the administration of antiidiotypic antisera to neonatal and adult mice (Hart et al., 1972). Because it has been postulated that immune regulation may be mediated by the interaction of idiotypes and antiidiotypes, forming a "network" (Jerne, 1974), the structural basis of idiotypy has been intensively addressed. Amino acid sequence analysis of a group of Id^{CR+} monoclonal antibody heavy and light chain variable regions identified sequence differences in both framework and CDR regions (Marshak-Rothstein et al., 1980; Estess et al., 1979, 1980; Margolies et al., 1981). Nonetheless, the sequences were highly homologous. Siekevitz and co-workers (Siekevitz et al., 1982, 1983) demonstrated that a single germ-line gene encoded the Id^{CR} V_H family. The DNA and protein structural studies were consistent with a large family of closely related but nonidentical V_H regions arising by somatic mutation from a single germ-line gene.

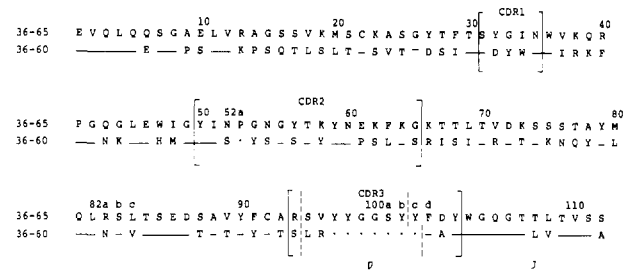

FIGURE 5: Amino acid sequence of the 36-60 heavy chain variable region compared to the sequence of the 36-65 heavy chain. Both proteins are anti-azophenylarsonate monoclonal antibodies derived from the A/J strain of mice. However, hybridoma 36-65 bears the predominant idiotype (Id^{CR}) (Kuettner et al., 1972), while hybridoma 36-60 is representative of a minor idiotype ($Id^{36\text{-}60}$) found in virtually all A/J mice (Marshak-Rothstein et al., 1981). The sequence of 36-65 was reported previously (Siekevitz et al., 1982; Marshak-Rothstein et al., 1980) and is identical in its V_H-encoded portion to the germ-line gene sequence for the Id^{CR} (Siekevitz et al., 1982, 1983). A line in the 36-60 sequence indicates identity with the residues in the 36-65 sequence. A dot represents a deletion relative to the 36-65 sequence. Complementarity-determining regions (CDR) are indicated in brackets. Regions encoded by the V_H, D, and J_H genes are separated by dashed vertical lines. The numbering is according to Kabat et al. (1979).

The existence of a second idiotype family ($Id^{36\text{-}60}$) among A/J anti-Ars hybridoma proteins was detected by serological and structural studies of randomly selected hybridoma proteins lacking the predominant Id^{CR} (Marshak-Rothstein et al., 1981). Both heavy and light chains from three of eight Id^{CR-} monoclonal antibodies were highly homologous (>95%) to each other in their partial V-region amino acid sequence. A rabbit antiserum prepared against one of these (hybridoma protein 36-60) cross-reacted extensively with the other two but with no other Id^{CR-} nor any Id^{CR+} hybridoma protein. In addition, it was demonstrated that this second idiotype family ($Id^{36\text{-}60}$) is present in virtually all A/J anti-Ars sera. Partial V_H sequences probably belonging to the $Id^{36\text{-}60}$ family have also been reported from other laboratories (Estess et al., 1980; Alkan et al., 1980). Not only are Id^{CR} and $Id^{36\text{-}60}$ serologically and structurally distinct antibody populations but also they are regulated independently, based on suppression experiments (Marshak-Rothstein et al., 1981). In Figure 5, the V_H-region sequences of the two A/J Ars-associated idiotypes (Id^{CR} and $Id^{36\text{-}60}$) are compared. The sequence of the Id^{CR+} hybridoma 36-65 is identical in its V_H-encoded portion with the germ-line gene sequence for the predominant idiotype Id^{CR} (Siekevitz et al., 1982, 1983). Although both monoclonal antibodies 36-65 and 36-60 were elicited by immunization with Ars–KLH, they are only homologous to an extent of 45% in the V region. Differences abound not only in framework regions but also in the CDR, including differences in chain length. Hybridoma 36-60 is shorter by one residue in CDR2 and by seven residues in CDR3 in comparison to hybridoma protein 36-65. In addition, the available V_L-region sequences of these two idiotypes also differ significantly from each other (Margolies et al., 1981; Marshak-Rothstein et al., 1981). Further V_H and V_L diversity among anti-Ars monoclonal antibodies was demonstrated in structural studies on hybridomas lacking either idiotype (Margolies et al., 1981; Milner & Capra, 1982).

The marked differences in V_H sequence between Id^{CR} and $Id^{36\text{-}60}$ heavy chains are in agreement with the serologic and functional studies indicating that they are encoded by separate germ-line V_H genes. R. Near and M. Gefter (unpublished results) have determined the A/J germ-line gene DNA sequence for $Id^{36\text{-}60}$. The amino acid sequence of the hybridoma protein 36-60 differs from the germ-line gene sequence at only

two positions in V_H (positions 47 and 93; Figure 5), each of which is accounted for by a single nucleotide base change. The marked degree of homology among all members of the Id^{36-60} V_H family sequenced to date is consistent with the Id^{36-60} family arising by somatic mutation from a single germ-line gene, analogous to the results for Id^{CR} (Siekevitz et al., 1982, 1983).

The 36-65 and 36-60 heavy chains differ not only in the V_H-encoded portions but also in the D and J_H regions as well. The 36-65 heavy chain contains the D-gene core sequence (Y-Y-G-G-S-Y), which corresponds to a combination of the D genes FL16.2 and Sp2.3 reported for BALB/c mice by Kurosawa & Tonegawa (1982). The D-gene sequence utilized by 36-60 was deduced from the known A/J germ-line V_H sequence for 36-60 (R. Near and M. Gefter, unpublished results) and the known J_{H3} sequence (Sakano et al., 1980). The 36-60 D-gene sequence is much smaller than the 36-65 D-gene sequence, corresponding to a portion of the D-gene FL16.2 (Figure 5). In all Ars-binding Id^{CR+} cell lines, the same germ-line V_H gene is rearranged to the J_{H2} segment (see portion 36-65; Figure 5) (Siekevitz et al., 1982, 1983; Sakano et al., 1980). In DNA hybridization studies (R. Near and M. Gefter, unpublished results), all Id^{36-60} hybridomas use J_{H3}, consistent with the protein sequence of hybridoma 36-60 reported here (see Figure 5). Thus, for two different idiotypes associated with anti-arsonate antibodies, the heavy chain variable regions utilize different genes in all three germ-line-encoded segments (V_H, D, and J_H). Further correlation between serology and sequence data from the Id^{CR} and Id^{36-60} families is necessary before the relevant idiotypes may be localized to one or more of these gene segments (Capra et al., 1982; Margolies et al., 1983).

The major A/J Ars-associated idiotype (Id^{CR}) is not expressed in BALB/c mice. However, among a heterogeneous population of minor Ars-associated idiotypes in A/J mice described by Nisonoff and co-workers (Gill-Pazaris et al., 1979; Brown & Nisonoff, 1981; Brown et al., 1981; Nelles & Nisonoff, 1982), one proved related to an idiotype (CRI_c) which predominated in the anti-Ars response in BALB/c mice. Id^{36-60}, a cross-reactive idiotype in the A/J strain, is also a cross-reactive idiotype in BALB/c mice (Marshak-Rothstein et al., 1981). Id^{36-60} was defined by antisera raised against the monoclonal anti-Ars antibody 36-60. $IdCRI_c$ was defined by antisera raised against *serum* anti-Ars antibodies and might include other idiotype families, in addition to Id^{36-60}. On the basis of the structural and serological data, it was predicted (Marshak-Rothstein et al., 1981) that the Id^{36-60} family present in both A/J and BALB/c strains is derived from the same or very similar germ-line genes shared by the two strains. Structural studies on a BALB/c anti-Ars hybridoma bearing Id^{36-60} support this contention (E. Juszczak, R. Near, and M. Margolies, unpublished results).

The high frequency of the Id^{CR} and Id^{36-60} idiotype families among A/J anti-Ars antibodies suggested that both groups resulted from the direct expression of germ-line genes (Kuettner et al., 1972; Marshak-Rothstein et al., 1981). However, Id^{36-60} is present only to an extent of 5–20% of anti-Ars antibodies, as compared to approximately 50% for Id^{CR} in A/J sera. One explanation for the predominance of Id^{CR} in A/J mice is that Id^{CR} exists as multiple gene copies. However, since the Id^{CR} family has now been demonstrated to arise from a single germ-line V_H gene (Siekevitz et al., 1982, 1983), a difference in gene number is not responsible for its predominance. A second possibility is that Id^{36-60} antibodies have lower affinities for antigen than Id^{CR}. This explanation is not supported by experimental evidence which indicates that the affinity of the Id^{CR+} hybridoma protein 36-65 is lower than that of the three representative Id^{36-60} hybridoma proteins (Rothstein & Gefter, 1983). Whether other control mechanisms are involved in the regulation of expression of these two idiotypes (Brown et al., 1981) has not been established.

Acknowledgments

We thank Ann Marshak-Rothstein, Richard Near, and Malcolm Gefter for supplying the 36-60 cell line and for helpful discussions. We are grateful to Andrew Brauer and Christine Oman for technical assistance and to Rose Hand for secretarial assistance.

Registry No. Immunoglobulin G2a (mouse hybridoma 36-60 V_γ protein moiety reduced), 86391-83-5.

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Rapid Suppression of α_1-Fetoprotein Gene Transcription by Dexamethasone in Developing Rat Liver†

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ABSTRACT: The administration of glucocorticosteroid hormones to newborn rats interrupts selectively (and reversibly, if the hormone is withdrawn) the hepatic production of α_1-fetoprotein (AFP). This results from a decreased concentration of AFP mRNA in the liver [Bélanger, L., Frain, M., Baril, P., Gingras, M. C., Bartkowiak, J., & Sala-Trepat, J. M. (1981) *Biochemistry 20*, 6665]. We have delineated further the mechanism and time course of this hormonal action in 4-day-old rats treated with dexamethasone (DEX). DNA from a recombinant plasmid containing a 578-bp insert of rat AFP cDNA was used to develop a cell-free nuclear run-off system and directly assess AFP gene transcription activity. Five minutes after DEX injection, AFP gene transcription activity is unchanged, but after 30 min, it drops to 25% that of the control; this correlates with the time required for translocation of DEX receptors to the nucleus. Dose–response curves also show that the degree of AFP gene suppression is closely correlated with the amount of DEX receptor translocated to the nucleus. The nuclear concentration of AFP mRNA, monitored by dot–blot hybridization, decreases to undetectable levels within 48 h, whereas that of albumin mRNA increases slightly, which indicates the selectivity of DEX action. These results show that DEX suppresses AFP gene expression at the transcriptional level and suggest a direct negative action of DEX–receptor complexes on the AFP chromatin transcription unit.

Studies on the regulation of eukaryotic gene expression have shown that steroid hormones can modulate the transcription of specific genes. Many model systems currently used to decipher the mechanisms of this action are based on positive modulations of gene functions. Only a few systems negatively modulated by steroids are exploitable at the molecular level. Such systems may provide valuable conceptual and experimental counterparts to the positive models.

One negatively regulated steroid-responsive gene system is the suppression of α_1-fetoprotein (AFP)[1] expression by glucocorticoid hormones in the developing liver. AFP is a fetal albumin, suppressed in adult liver but reexpressed in hepatocarcinomas. The administration of dexamethasone to developing animals suppresses prematurely the hepatic production of AFP (Bélanger et al., 1975, 1978, 1983). This action is nontoxic, selective, and reversible if hormone is withdrawn. We have previously reported (Bélanger et al., 1981) that following DEX injection to newborn rats, the levels of AFP mRNA decrease exponentially in the liver, with kinetics extrapolating to near time of injection; this suggested a rapid transcriptional effect on the AFP gene. Here, we confirm and extend this interpretation by showing, in a cell-free nuclear transcription system, that DEX suppresses AFP gene transcription within 30 min and that this action is time- and dose-related to the accumulation of DEX receptors in liver nuclei.

Experimental Procedures

Animals and Hormone Treatment. Four-day-old Sprague-Dawley rats were used. Half of each litter received intraperitoneal injections of dexamethasone (DEX) (Decadron; Merck Sharp & Dohme) at 2 μg/g twice a day in 50 μL of

† From the Laval University Cancer Research Center at l'Hôtel-Dieu de Québec, Québec, Québec, Canada G1R 2J6. *Received March 15, 1983.* This work was supported by grants from the Medical Research Council and the National Cancer Institute of Canada. M.G. was supported by a postdoctoral fellowship from the National Cancer Institute of Canada, P.B. by a studentship from the National Cancer Institute of Canada, J.B. by a postdoctoral fellowship from the Montreal Cancer Research Society Inc., and L.B. by a scholarship from the Medical Research Council of Canada.

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[1] Abbreviations: AFP, α_1-fetoprotein; DEX, dexamethasone; BSA, bovine serum albumin; SSC buffer, 0.15 M NaCl–0.015 M sodium citrate buffer; Hepes, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane; TCA, trichloroacetic acid; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; NaDodSO$_4$, sodium dodecyl sulfate; APT-cellulose, (aminophenyl thioether)-cellulose; DPT-cellulose, (diazophenyl thioether)-cellulose.

0006-2960/83/0422-4296$01.50/0