

EVIDENCE FOR THE LINKAGE OF THE IgC_H LOCUS TO A GENE
CONTROLLING THE IDIOTYPIC SPECIFICITY OF
ANTI-*p*-AZOPHENYLARSONATE ANTIBODIES IN
STRAIN A MICE*

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Rabbit anti-idiotypic antisera specific for anti-*p*-azophenylarsonate (anti-Ar)¹ antibodies, raised in an individual strain A/J mouse, recognize determinants on anti-Ar antibodies induced in all members of this strain so far tested. These determinants have been found only on anti-Ar antibodies and not on normal immunoglobulins or on other anti-hapten or anti-protein antibodies induced in this strain (1-3).² The determinants on A/J anti-Ar antibodies are thus idiotypic and cross-reactive within the strain. In contrast, anti-Ar antibodies induced in most but not all other strains of mice (1, 3; Pawlak and Nisonoff, manuscript in preparation), lack the cross-reactive A/J idiotypic determinant. For this reason the idio type of A/J anti-Ar antibody is potentially a genetic marker (phenotype).

We report here finding the A/J idio type on anti-Ar antibodies induced in strain AL/N mice; this strain was derived from an outcrossed strain A mouse (4). Strains AL/N and A/J share a number of characteristics, including similar *H*-2 complex loci (4) and most but not all IgC_H allotypic determinants (5, 6), which are localized to the Fc fragment of the molecule. Strain AL/N then probably shares many genes with strain A/J but, according to Staats (4) cannot be considered a true subline of strain A (4).

The presence of the cross-reactive idiotypic determinants on anti-Ar antibodies induced in strain AL/N mice is particularly fortuitous because we have recently completed, at the National Institutes of Health, the development of a congenic strain of BALB/c mice, AL/N IgC_H, in which the IgC_H complex locus from AL/N mice was introgressively backcrossed onto BALB/c. This

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¹ Abbreviations used in this paper: anti-Ar, anti-*p*-azophenylarsonate antibodies; anti-PC, anti-phosphorylcholine BGG, bovine gamma globulin (fraction II); KLH, keyhole limpet hemocyanin; RGG, rabbit IgG.

² Pawlak, L. L., A. L. Wang, and A. Nisonoff. 1972. Manuscript submitted for publication.

new congenic stock has been backcrossed for 20 consecutive generations and a strain homozygous for the AL/N IgC_H allotypic locus is currently being developed. However, before this we developed an earlier stock at the ninth backcross (BALB/c.AL/N IgC_H BC9 F3 to 5).³ The mice of this stock are homozygous for the AL/N IgC_H complex locus. We report here the results of quantitative measurements of idiotypic specificities of anti-Ar antibodies developed in the BC9 congenic mice. The data are discussed in terms of the linkage of genes controlling idiotypic specificities to genes controlling the IgC_H locus. Accounts of these results have been presented elsewhere (3, 6 a).

Materials and Methods

Myeloma Proteins.—Myeloma proteins were obtained from plasmacytomas induced in the BC9 homozygous mice by three intraperitoneal injections of pristane (7).

Determination of H-2 Type of the BC9 Mice.—The H-2 type of homozygous congenic BC9 mice of the F₃ generation was kindly determined by Dr. Jack H. Pincus of the National Institute of Dental Research, who used a microcytotoxicity assay method (8), which employs a B10.D2 × C3H.NB anti-LP.RIII antiserum. The antiserum, which is specific for H-2 determinants 11 and 25, gave a positive reaction with A/J cells when diluted 1/128 and was negative for DBA/2 and for the BC9 mice at various dilutions. Thus the congenic mice must be of the H-2 type of the BALB/c background strain.⁴

Immunization of Mice.—Methods for preparation of the antigens used for immunization and testing have been described (1). Anti-*p*-azophenylarsonate antibodies used to elicit anti-idiotypic antibodies were produced in A/J mice, nos. 413 and 126, by immunization with keyhole limpet hemocyanin (KLH)-*p*-azophenylarsonate. The immunization schedule comprised four weekly injections of 0.5 mg KLH-*p*-azophenylarsonate in Freund's complete adjuvant with subsequent periodic inoculation of 0.5 mg KLH-*p*-azophenylarsonate in Freund's incomplete adjuvant as necessary, i.e., whenever the antibody titer dropped markedly. In both mice the method of Sommerville (9) was used to produce an ascites fluid, which was used as a source of anti-hapten antibody. Hyperimmune sera were also employed in many experiments.

*Anti-Idiotypic Antisera Directed to Anti-*p*-Azophenylarsonate Antibodies of A/J Mice.*—Anti-idiotypic antisera to the anti-phenylarsonate antibodies of individual mice were prepared in rabbits as previously described (1, 2). In brief, the method consists of precipitation of the anti-hapten antibody with rabbit IgG (RGG)-*p*-azophenylarsonate (mouse 413) or bovine gamma globulin (BGG)-*p*-azophenylarsonate (mouse 126) in the presence of 0.01 M ethylenediaminetetraacetate, dissolution of the precipitate at pH 3.5, and emulsification in Freund's complete adjuvant. 1 mg of anti-hapten antibody was used for each injection. The resulting rabbit antiserum was absorbed with IgG and normal serum from A/J mice (1).

Quantitative Assay of Idiotypic Antibodies.—Specifically purified anti-*p*-azophenylarsonate antibodies (1) from the donor mouse, which provided the immunogen used for eliciting anti-idiotypic antibody, was trace labeled with ¹²⁵I by the chloramine-T method (10). The assay system comprised 0.01 μg of labeled antibody, 50 μl of a 1:100 dilution of the rabbit anti-idiotypic antiserum, 25 μl of a 1:10 dilution of normal rabbit serum, and an excess (80 μl)

³ The symbol BC9 indicates nine backcrosses; F3 to 5 indicates the number of generations of continued inbreeding of the homozygous congenic mice. In referring to these mice we will use the abbreviation BC9. These congenic mice are highly susceptible to the induction of plasmacytomas by mineral oil.

⁴ The H-2 histocompatibility types of DBA/2 and BALB/c mice are identical.

of goat antiserum to mouse IgG; the antiserum had been absorbed with normal A/J serum. The anti-idiotypic serum, present in slightly less than an optimal quantity, was incubated with the labeled ligand for 1 h at 37°C before addition of goat anti-rabbit Fc.

After incubating for another hour at 37°C, mixtures were allowed to stand for 8–16 h in the cold before centrifugation and washing of precipitates and determination of the percentage of radioactivity precipitated (1). Unlabeled inhibitors, when present, were incubated with the anti-idiotypic antiserum for 15 min at 37°C before addition of the labeled ligand.

Controls were carried out in each series of experiments by using rabbit anti-ovalbumin in place of the anti-idiotypic antiserum. The small percentages of radioactivity precipitated (6–8%) were subtracted from experimental values.

Specificity of Anti-Idiotypic Antibodies.—Evidence for specificity of these antisera has been described (1, 3). It includes the lack of inhibitory capacity of preimmune serum from the donor mouse, or of its hyperimmune serum after removal of anti-hapten antibody by immune adsorption. The adsorbed antisera did not cause significant inhibition of binding of ^{125}I -labeled ligand (specifically purified anti-Ar antibody of the donor mouse) by anti-idiotypic antibody. Anti-idiotypic antisera did not bind significant percentages of ^{125}I -labeled nonspecific mouse IgG. The preparation of the immunoadsorbent, BGG-*p*-azophenylarsonate conjugated to Sepharose beads, has been described (1).

RESULTS

*Idiotypic Cross-Reactions of Anti-*p*-Azophenylarsonate Antibodies of Different A/J Mice.*—As shown elsewhere (1, 2) anti-Ar antibodies of different A/J mice show idiotypic cross-reactions. The anti-idiotypic antiserum used in those studies, which was prepared against the anti-Ar antibodies of mouse 413, has been described (1).

Similar experiments were carried out with the other anti-idiotypic antiserum, directed to the anti-Ar antibodies of mouse 126. Each of 15 A/J hyperimmune anti-Ar antisera tested was capable of displacing at least 75% of the ^{125}I -labeled purified anti-Ar antibody of mouse 126 from its anti-idiotypic antibodies. The weight of precipitating anti-Ar antibody required for 50% displacement of 0.01 μg of the labeled ligand varied from 0.03 to 0.38 μg , with a median value of 0.08 μg . Evidence that antiserum 126 is specific for idiotypic determinants on the anti-Ar antibodies has been presented elsewhere (3).

Reactions of Anti-Idiotypic Antibodies with Anti-Phenylarsonate Antibodies of Other Strains of Mice.—Hyperimmune antisera from mice of various strains, immunized with KLH-*p*-azophenylarsonate, have been tested for their capacity to inhibit the binding of ^{125}I -labeled specifically purified anti-*p*-azophenylarsonate antibodies from A/J mice, nos. 126 and 413, to their respective anti-idiotypic antibodies. These results will be published elsewhere.⁵ Only antisera from strains very closely related to A/J, as indicated by their allotype and histocompatibility type, were found to be inhibitory. For the purpose of the present investigation it is germane that one of the strains showing strong idiotypic cross-reactions is AL/N, and that the anti-Ar antibodies of BALB/c mice do not cross-react. Data concerning these cross-reactions will be presented

⁵ Pawlak, L. L., and A. Nisonoff. Manuscript submitted for publication.

below. On a quantitative basis the anti-Ar antibodies of AL/N mice are roughly one-half as effective as inhibitors, with each of the two anti-idiotypic antisera tested, as the anti-Ar antibodies of various A/J mice. These are average values since individual mice differ considerably.

Idiotypic Cross-Reactions of Anti-Ar Antibodies Elicited in Congenic Mice.—

As a result of these observations it was possible to ask the question whether the congenic mice, bearing the heavy chain allotype of AL/N mice on a BALB/c background (see Methods), showed idiotypic cross-reactions characteristic of AL/N or BALB/c mice. The results, obtained with the hyperimmune anti-Ar antisera of eight congenic mice and with two different anti-idiotypic antisera, are shown in Figs. 1 and 2. Also presented in these figures are the data obtained with anti-Ar antisera of 10 or 14 BALB/c mice and 9 or 15 AL/N mice.

It is apparent that anti-Ar antibodies of the congenic mice are, on the average, as effective as inhibitors of the anti-idiotypic antibodies as anti-Ar antibodies of AL/N mice. Antibodies from BALB/c mice had little or no inhibitory

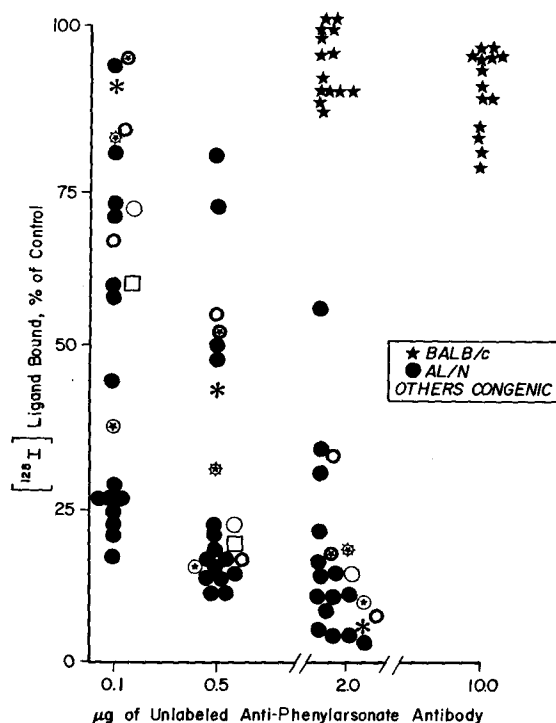


FIG. 1. Inhibition of binding of 0.01 µg of ^{125}I -labeled purified anti-Ar antibody of A/J mouse 126 to its rabbit anti-idiotypic antibodies. Unlabeled inhibitors are anti-Ar antisera from BALB/c, from AL/N, or from BC9 (congenic) mice containing the amount of precipitable anti-Ar antibody indicated on the abscissa. Each symbol represents the serum of an individual mouse, which was tested at two or three concentrations.

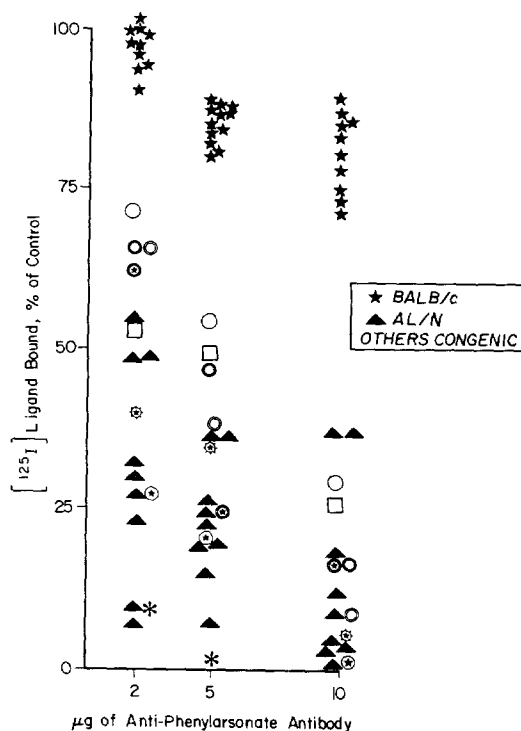


FIG. 2. The legend is the same as that of Fig. 1, except that reaction mixtures comprised ^{125}I -labeled anti-Ar antibody of mouse 413, reacting with its anti-idiotypic antibodies.

effect. On a quantitative basis the antibodies of the congenic mice were more than 10 times as effective as the BALB/c antibodies (Figs. 1 and 2). This is a minimum value since BALB/c antibodies did not cause 50% inhibition at the highest concentration tested.

Each anti-Ar antiserum from AL/N or congenic mice was tested after removal of anti-Ar antibodies on Sepharose conjugated to BGG-*p*-azophenylarsonate. In each case, all inhibitory capacity was removed by the immuno-adsorbent.

Absence of the Cross-Reactive Idiotypic in Myeloma Proteins Raised in the Congenic Mice.—Plasmacytomas were prepared in BC9 congenic mice by intraperitoneal injections of pristane (7). Ascites fluids from 15 such mice, each containing a high concentration of a myeloma protein (6 IgA, 4 IgF, 1 IgG, 1 IgG3, 3 IgH) were tested for the A/J anti-Ar idiotype of mouse 126 by the inhibition assay, using 50 μl of ascites fluid and 0.01 μg of labeled ligand per test. No inhibitory activity (<10% in each case) was observed.

Presence of Cross-Reactive Idiotypic in F_1 Mice.—Anti-Ar antibodies were elicited in two F_1 strains of mice, C57 \times A/J (16 mice) and BALB/c \times A/J

(21 mice). The cross-reactive idiotypic, causing 30–80% displacement, was found in varying concentrations in all but four of these mice. About half of the sera in each F_1 strain were equivalent to A/J sera in inhibitory potency. All C57/BL mice tested, as well as BALB/c, failed to express the idiotypic.

DISCUSSION

The principal finding reported in this paper is that anti-Ar antibodies raised in strain AL/N mice and in the congenic strain, BALB/c.AL/N IgC_H BC9 (abbreviated BC9), share a cross-reacting idiotype while antibodies raised by a similar procedure in BALB/c mice do not. Since the BC9 stock was made by introgressively backcrossing the IgC_H complex locus of strain AL/N mice onto the BALB/c background, the findings presented in this paper indicate that genes controlling the anti-Ar idiotypic are linked to the IgC_H locus, which controls allotypic determinants in the Fc regions of the heavy chains of mouse immunoglobulins. The absence of the anti-Ar idiotypic on anti-Ar antibodies induced in many other strains makes the finding of this idiotypic in AL/N and in the BC9 mice even more significant. The only strains, other than A/J, in which anti-Ar antibodies carrying the cross-reacting idiotypic have been inducible are the closely related strains AL/N, A/He, and A/WySn and congenic strains derived from A/J by introgressively backcrossing different *H-2* genes (A.SW, A.BY).⁵

In quantitative terms there was no significant difference between the capacities of the anti-Ar antibodies of congenic and AL/N mice to displace labeled anti-Ar antibodies of A/J mice from their anti-idiotypic antibodies. Similar results were obtained with two such anti-idiotypic antisera (Figs. 1 and 2). The anti-Ar antibodies of both the congenic and AL/N strains were far more effective as inhibitors than antibodies of the same specificity from BALB/c mice, which failed to cause significant displacement even at much higher concentrations. It is apparent that the gene(s) controlling idiotype specificity are linked to the genes which control the allotypes of heavy chains (localized in the C_H region).

We will discuss the data on the basis of the hypothesis that two genes control the biosynthesis of a single immunoglobulin polypeptide chain. The evidence supporting this view, which was introduced by Dreyer and Bennett, (11) has been summarized in a recent symposium (12).

On this basis, the simplest interpretation of our results is that the V_H gene, which in all probability is involved in the determination of idiotype specificity, is closely linked to the C_H gene, since the two genes failed to segregate during nine backcross generations. This result is in accord with studies of allotypes of rabbit IgG in which it has been shown that the *a* locus, which evidently controls determinants in the V_H region (13), is closely linked to the *d* (14, 15) and *e* (16, 17) loci, which control allotypic markers in the constant portion of the heavy chain.

It is unclear why the idiomorph of AL/N mice was fully expressed in the congenic mice despite the fact that selection during backcrossing involved only genes controlling the C_H regions and did not select for AL/N genes controlling light chain biosynthesis. At least three possibilities may be considered to account for this result. (a) The idiomorph of anti-Ar antibody is completely determined by the heavy chain. Although this is uncommon it has been observed in certain human myeloma proteins (18). The possibility is being investigated. (b) The genes controlling biosynthesis of the light chain and of the constant region of the heavy chain are closely linked; in this case the congenic mice would synthesize light chains, as well as heavy chains, characteristic of AL/N mice. If so, the mouse differs in this respect from the rabbit and man, since genes controlling heavy and light chains are unlinked in those two species. (c) BALB/c mice may be capable of producing light chains identical with those present in the anti-Ar antibodies of AL/N mice. In this event, congenic BC9 mice could synthesize anti-Ar antibodies bearing the cross-reacting idiomorph by using heavy chains encoded by AL/N genes and light chains encoded by BALB/c genes. This possibility does not seem remote since the light chains of different strains of mice have not as yet been distinguished by anti-allotypic antisera (19-21), although some evidence of allotypic variation has been obtained through peptide mapping (22).

Evidence for linkage of genes controlling the idiomorph of anti- α -1,3-dextran antibodies and the heavy chain allotype has been recently reported by Blomberg et al. (23). Anti- α -1,3-dextran antibodies raised in BALB/c mice carry an idiomorph that cross-reacts with anti-idiotypic antiserum directed to a homogeneous BALB/c myeloma protein (J558) which binds α -1,3-dextran, while antibodies raised in C57BL mice lack the idiomorph. Since anti-idiotypic antibodies were prepared by immunization with the J558 myeloma protein, the cross-reacting determinant is referred to as the J558 idiomorph. Blomberg et al. (23) found evidence for linkage of the gene controlling the J558 idiomorph with the BALB/c IgC_H complex locus, i.e., with genes controlling heavy chain allotypes. This was based on the distribution of the J558 idiomorph in the Bailey recombinant strains (24). These strains were developed by Dr. Donald Bailey of The Jackson Laboratory from a cross between C57BL/6 and BALB/c. Essentially, seven pairs of F₂ hybrid mice from this cross were randomly selected and inbred for 20 generations. The seven new inbred strains so derived contain various mixtures of C57BL and BALB/c genes. Two of the strains, C \times BG and C \times BJ, carry the BALB/c IgC_H complex locus and, in these two strains only, Blomberg et al. found that the anti- α -1,3-dextran antibodies contained the J558 idiomorph. Since this distribution pattern among the seven strains is one of 128 different possible patterns the result strongly suggests that the J558 idiomorph and the IgC_H locus are linked.

Because expression of the characteristic anti- α -1,3-dextran idiomorph in BALB/c mice requires participation of both heavy and light chains, Blomberg

et al. concluded that the gene controlling the light chain (λ -type) of the BALB/c anti- α -1,3-dextran antibody must be linked to the gene controlling the C_H region of the antibody. (Mice were selected during breeding for heavy chain allotypic determinants, which are localized in the C_H region.) As an alternative possibility, not requiring linkage of genes controlling the light and heavy chains, we would suggest that the C57 strain might be capable of synthesizing λ -chains with a structure identical with those of BALB/c; such λ -chains might be utilized in combination with BALB/c heavy chains to form anti-dextran antibodies with the BALB/c idiotype.

One observation made by Blomberg et al. (24) appears to conflict with the data obtained with the recombinant inbred mice. They also tested mice from an Ig-congenic stock made by introgressively crossing C57BL/Ka Ig C_H genes onto BALB/c (6). They used for this purpose BAB-14, which is a homozygous stock derived from BALB/c.C57BL/Ka Ig C_H BC14. BAB-14 was made homozygous by Dr. Leonard Herzenberg of Stanford University and has been brother-sister mated to produce an inbred line. BAB-14 mice are homozygous for the C57BL/Ka Ig C_H complex locus but surprisingly produced anti- α -1,3-dextran antibodies with the J558 idiotype (24). This was attributed to a cross-over between the Ig C_H and IgV H loci. Similar crossovers have been observed in rabbits (25, 26). It was not specified whether such a crossover must have occurred in a number of matings during backcrossing or whether it could have been a single event.

It is evident from these and previous investigations (1-3, 23) that idiotypic specificity provides a convenient phenotypic marker for the variable region of immunoglobulin polypeptide chains. More precise information on linkage between V H and C_H genes, and between C_H genes and those controlling light chain biosynthesis, requires further investigation. Congenic mice (BALB/c.AL/N) resulting from a larger number of backcrosses will soon be available; experiments with these mice should provide additional data on the closeness of linkage of V and C genes.

The presence of the cross-reactive idiotype in nearly all F_1 mice tested indicates that the failure to observe the idiotype in the BALB/c or C57/BL strains is not due to a suppressive factor present in those strains.

SUMMARY

Anti-*p*-azophenylarsonate (anti-Ar) antibodies elicited in all strain A/J mice tested share one or more idiotypic specificities. These specificities are also found in the anti-Ar antibodies of mice of the closely related strain, AL/N, but not in those of BALB/c mice.

Anti-Ar antibodies were elicited in congenic mice in which the Ig C_H locus of AL/N mice, which controls allotypic markers in the constant regions of heavy chains, had been introgressively backcrossed for nine generations onto a BALB/c background; the mice were then rendered homozygous for the AL/N

allotypic determinant. On the average, these antibodies were quantitatively equivalent, with respect to content of the cross-reactive idiotypic, to those of AL/N mice. This indicates that the gene controlling the idiotypic is closely linked to the IgC_H locus. Since idiotypic must be a function of V region sequences, the results suggest close linkage of V_H and C_H genes. The cross-reactive idiotypic was found in nearly all F₁ mice (C57/BL × A/J or BALB/c × A/J) tested.

Note Added in Proof.—Sher and Cohn (27) have recently shown that two congenic mice, bearing the heavy chain allotype of the AL/N strain on a BALB/c background, produced anti-phosphorylcholine (anti-PC) antibodies, some of which were not reactive with an anti-idiotypic antiserum that inhibits nearly all anti-PC plaques produced very early in immunization of BALB/c mice. This finding would indicate either that there is close linkage between the AL/N allotype and the idiotypic expressed, i.e. an idiotypic characteristic of AL/N mice, or, alternatively, that the congenic strain produces anti-PC antibodies of BALB/c origin, some of which possess other, noncross-reacting anti-PC idiotypes, which have been observed in BALB/c mice (28).

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