Brief Definitive Report

GENETIC POLYMORPHISM OF MOUSE IMMUNOGLOBULIN LIGHT CHAINS REVEALED BY ISOELECTRIC FOCUSING*

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One of the key objectives in current research on the genetic control of antibody structure is the localization of genetic markers in the variable region of immunoglobulin heavy and light chains (1). Not only do such markers provide a means of estimating the number of genes encoding variable region structure, but they also provide a much needed probe for studying the arrangement and control of variable region genes. One approach to this problem which has met with considerable success has been the development of procedures for detecting specific *v*-region sequences using anti-idiotype antisera. These procedures have led to the discovery of a number of *v*-region-associated genetic markers in the heavy chain of mouse immunoglobulin (2, 3). A more direct approach was that described by Edelman and Gottlieb (4) who used peptide mapping of [^{14}C]iodoacetate-labeled light chains to show genetically controlled structural differences in the variable region of mouse light chains. Their results indicated that the light chains of certain strains of mice showed an additional radioactive peptide (I_B) when analyzed by this technique. More recently, Gottlieb has shown that the I_B marker associated with the mouse kappa chain *v* region is genetically linked to the *Ly-3* locus specifying a thymocyte surface antigen carried on chromosome 6 of the mouse (5).

In the present report a different approach has been used to examine the light chains of mouse immunoglobulin for evidence of genetic polymorphism. This approach has simply been to analyze the normal light chain pool of various inbred strains of mice by the technique of gel isoelectric focusing. This study has led to the discovery of a new polymorphism of mouse light chains involving the positions of several focusing bands. Preliminary reports of this work have appeared (6, 7).

Materials and Methods

Mouse Serum. Pooled serum of the following inbred strains of mice was obtained from The Jackson Laboratory, Bar Harbor, Maine: DBA/1J, A/J, CBA/J, C58/J, C57BL/6J, C3H/HeJ, RF/J, SWR/J, and AKR/J. In addition, serum from individual C58/J, SWR/J, and F_1 hybrid mice was obtained from animals raised in this laboratory.

Preparation of Immunoglobulin Fraction. Mouse serum was first diluted with an equal volume of 0.9% NaCl, 0.02 M potassium phosphate, pH 6.8 (phosphate-buffered saline, PBS). Twice the original serum volume of 3.5 M ammonium sulphate, pH 7.3 was then added with agitation. Precipitation was then allowed to proceed for 1 h at 4°C. After centrifugation the precipitate was redissolved in twice the original serum volume of PBS, and the precipitation was repeated. The centrifugation and precipitation steps were then repeated one more time (total of three precipitations). This procedure gave a highly reproducible product which contained more then 90% immunoglobulin as judged by acrylamide gel electrophoresis at pH 8.8.

Preparation of Light Chains by Sephadex Gel Filtration. The immunoglobulin fraction from 5

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ml of serum was dissolved in 2.5 ml of 0.2 M Tris-HCl pH 8.0 and subjected to partial reduction and alkylation with iodoacetamide. Heavy and light chains were separated on a column (2.5 × 100 cm) of Sephadex G-100 equilibrated with 1 M propionic acid. The light chain fraction was rechromatographed on a column of Sephadex G-100 equilibrated with 5 M urea, 0.5 M propionic acid. This procedure gave light chains which were greater than 95% homogeneous, as judged by electrophoresis in the presence of 0.1% sodium dodecyl sulphate (8).

Complete Reduction and Alkylation of Isolated Light Chains. Approximately 0.5 mg of light chain was dissolved in 0.1 ml of freshly prepared 8 M urea, 0.2 M Tris-HCl pH 8.2, 1 mM EDTA. The urea used for the buffer was freshly deionized by passage through a column of Amberlite MB-3 resin (Mallinckrodt Inc., St. Louis, Mo.). Initial reduction was carried out at 37°C for 30 min by the addition of 10 μl of dithiothreitol (DTT) (1.25 mg/ml in the urea buffer). Alkylation with [^{14}C]-iodoacetamide was then conducted at 37°C for 30 min by the addition of 10 μl of [^{14}C]iodoacetamide solution (60 Ci/mol, 500 mCi/ml, Amersham/Searle Corp., Arlington Heights, Ill.). Complete reduction and alkylation was then performed by successive additions of (*a*) 10 μl of DTT (15 mg/ml in urea buffer) and (*b*) 10 μl of iodoacetamide (40 mg/ml in urea buffer). In each stage the reaction was for 30 min at 37°C. The reaction was finally terminated by the addition of 10 μl of DTT (15 mg/ml in urea buffer). The final vol of the light chain solution was 0.15 ml. Approximately 20 μl of this solution was then applied to the isoelectric focusing gel in a small strip of Whatman no. 3MM filter paper (8 × 10 mm).

Isoelectric Focusing. Acrylamide gel isoelectric focusing (9) was conducted at 10°C using an LKB multiphor apparatus essentially as described previously (10). The gels contained 6 M urea, 2% ampholine (LKB Instruments, Rockville, Md.), 5% acrylamide, 0.13% *N,N'*-methylenebisacrylamide and 0.026 mg/100 ml riboflavin. Gels were stained with Coomassie blue R250 (Bio-Rad Laboratories, Richmond, Calif.) (11), dried using a photographic print dryer, and autoradiographed using Kodak RP-14 X-ray film.

Preparation of Light Chains by Urea-Formate Gel Electrophoresis. For analysis of light chains from individual mice a procedure was developed which required only 0.2 ml of serum. The first step was the preparation of the immunoglobulin precipitate as described above. The immunoglobulin fraction was then dissolved in 0.2 ml of 8 M urea, 0.2 M Tris-HCl, pH 8.2, 1 mM EDTA and subjected to complete reduction and alkylation in a two-step procedure similar to that described for reduction and alkylation of the light chains. After the reduction and alkylation step, the protein was precipitated free of urea and reagents by the addition of 2 vol of methanol at 0°C. The precipitate was collected and dissolved in 50 μl of 8 M urea, 0.05 M formic acid, 0.01 M Tris buffer for application to the urea-formate acrylamide gel.

Urea-Formate Gel Electrophoresis. Heavy and light chains from completely reduced and alkylated immunoglobulin could be separated effectively using the urea-formate gel system originally used with starch gel electrophoresis (12). The final composition of the gel used here was 7.5% acrylamide, 0.2% bisacrylamide, 8 M urea, 0.05 M formic acid, 0.01 M Tris. Polymerization was effected by the inclusion of 50 μl of *N,N,N',N'*-tetramethylethylenediamine and 50 mg of ammonium persulfate per 100 ml of gel solution. Electrophoresis was carried out using a Bio-Rad slab gel system Model 220, with a gel thickness of 1.5 mm and 10 samples per slab. For each run duplicate gels were run simultaneously. One gel was loaded with 5 μl of the reduced and alkylated immunoglobulin solution (preceding paragraph), while the second gel was loaded with 40 μl of the sample solution. Electrophoresis was carried out at 300 V for 5 h.

The position of heavy and light chain bands was identified by staining the analytical scale gel (5-μl samples) with Coomassie Blue (13). The portion of the gel containing the light chain was then cut from the preparative (40-μl sample) gel and placed directly on the surface of the isoelectric focusing gel. The entire surface of the isoelectric focusing gel was then covered with a layer of petrolatum (14) to prevent drying of the applied acrylamide gel strip. Isoelectric focusing was then commenced in the normal fashion, as described (10).

Results

The type of banding pattern obtained upon isoelectric focusing of normal light chains is seen in Fig. 1, which shows a comparison of the focusing patterns of light chains from nine different inbred strains of mouse. The first striking feature of the patterns was the virtual identity observed in several of the mouse

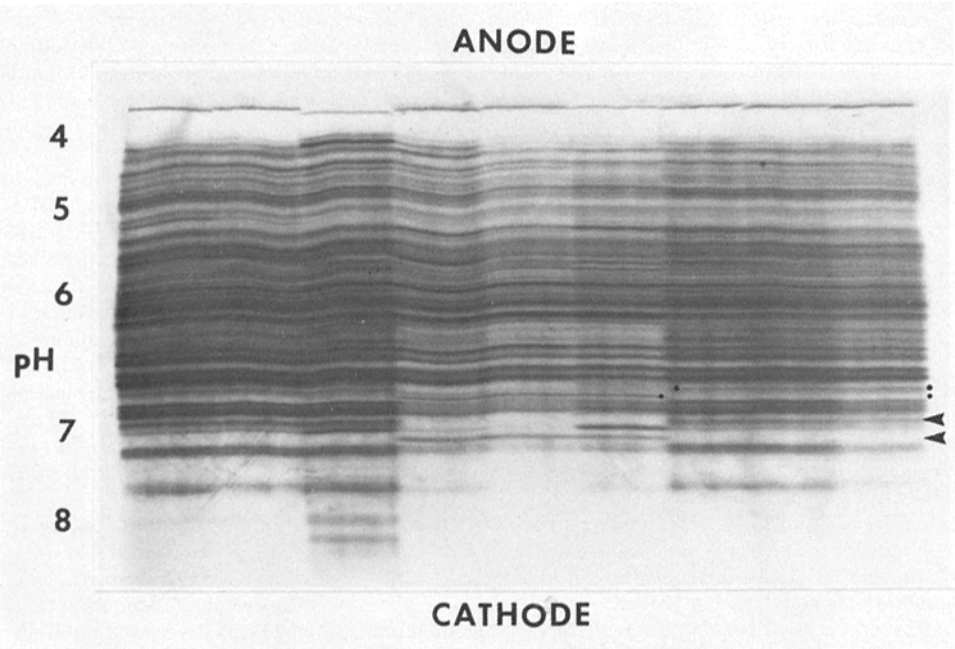

FIG. 1. Autoradiogram of [^{14}C]carboxymethylamido light chains of various inbred strains of mice. Left to right: DBA/1J, A/J, C57BL/6J, AKR/J, RF/J, C58/J, SWR/J, C3H/HeJ, and CBA/J. The isoelectric focusing gel contained 6 M urea, 2% carrier ampholytes (LKB, pH range 3.5–10), and 5% acrylamide. Arrows indicate the shared differences involving strains AKR/J, RF/J, and C58/J.

strains. This high degree of identity suggests that genetic factors must play a dominant role in governing the focusing pattern. While virtual identity was observed for six of the strains examined, clear differences were noted in the focusing patterns of the strains RF, AKR, and C58. All three of these strains possessed at least one band not present in the other strains and lacked another band that was present in all of the other strains (Fig. 1, arrows). In addition, other shared differences were also evident in the RF, AKR, and C58 patterns (Fig. 1, dots).

To establish whether the strain differences observed are genetically determined, F_1 hybrid mice obtained from the cross-C58 × SWR were examined. Fig. 2 shows a comparison of the focusing pattern of light chains from individual C58, SWR, and F_1 hybrid mice. From this result it was evident that the F_1 hybrid pattern contained all of the elements of both the parental banding patterns, indicating that the differences observed are transmissible and are expressed codominantly. The major differing bands are tentatively designated as IF-I, IF-II, IF-III, IF-IV, and IF-V. The phenotypes of the C58, SWR, and F_1 hybrid would thus be IF-I, III, IF-I, II, IV, V, and IF-I, II, III, IV, V, respectively.

Discussion

The present results indicate that genetic polymorphism can be clearly detected by isoelectric focusing analysis of normal mouse immunoglobulin light chains. The fact that the differences in the light chain focusing patterns in-

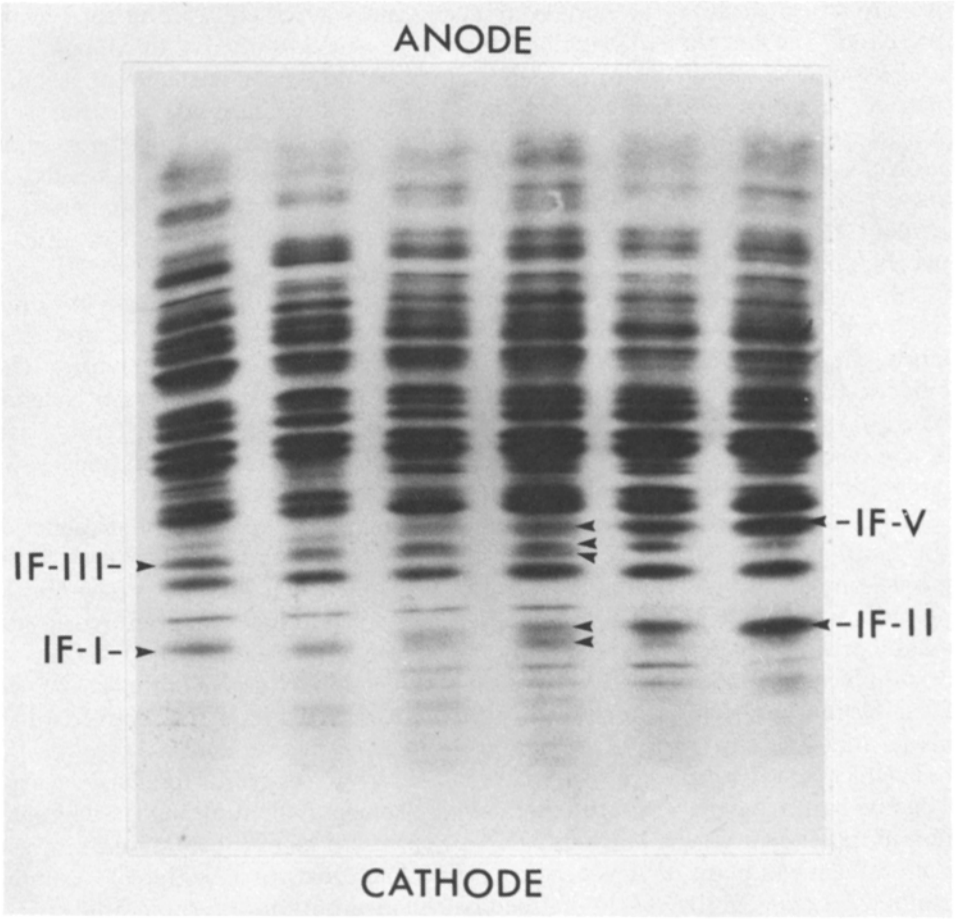

FIG. 2. Isoelectric focusing patterns of light chains from individual mice. Light chains were separated from heavy chains by electrophoresis on an 8 M urea-formate gel as described in the methods section. Samples, left to right: C58/J, C58/J, F_1 hybrid, F_1 hybrid, SWR/J, and SWR/J. Arrows indicate the parental bands expressed in a codominant fashion in the hybrid animals. Autoradiogram of the [^{14}C]carboxymethylamido light chain pattern.

volved a relatively small proportion of the total light chain population (roughly 5%) suggests that the polymorphism may be located in the variable region of the chain. Polymorphism of C-region structural genes would be expected to affect a much larger proportion of the light chain population. The possibility that the polymorphism may actually be localized in the C region of the minor lambda type of light chain (represented at about the 5% level in mouse [15]) cannot be ruled out at this time, however.

A number of intriguing questions are raised by the present observations. One of these concerns the relationship of the individual focusing bands to the light chain variable region subgroups observed in the mouse myeloma sequence data (17). A definitive answer to this question, however, must await the isolation and sequence analysis of the focusing bands. A related question pertinent to the

present work concerns the number of germ-line v genes controlling each focusing band. The fact that the strain differences seemed to involve the appearance and disappearance of entire focusing bands would argue that one or a small number of genes may control each band. This in turn suggests that the total number of germ-line genes controlling light chain structure may be a small multiple of the number of discrete focusing bands. The number of bands observed in the patterns illustrated in Fig. 1 was approximately 50, but it is evident that the real number will be considerably larger than this due to overlapping of bands.

The possibility that the differences observed are due to differences in enzymes involved in postsynthetic modification of certain light chains seems unlikely, since this effect would be expected to show dominance in the F_1 hybrid. The evidence presented here indicates that the differences are expressed codominantly. Although the simplest working hypothesis for the genetic interpretation of the results is that there is a polymorphism of structural *v*-region genes, the possibility that regulatory genes are involved cannot be ruled out.

A relationship between the isoelectric focusing phenotype and the presence of the I_B allele is suggested by the strain distribution of the two markers (4). In particular, strains possessing the bands IF-I and IF-III also possessed the I_B peptide. Isolation of the individual focusing bands will be attempted in order to establish whether light chains giving rise to the I_B peptide may be localized in certain focusing bands. An investigation of the possible linkage of the isoelectric focusing markers with other known biochemical markers of the mouse is also being undertaken.

The fact that the differences observed between mouse strains involved several focusing bands suggests that the method may be useful for studying the segregation of multiple *v*-region markers. This in turn will be of considerable interest from the genetic point of view because it will facilitate the detection of recombinants. The applicability of the methods developed here to the rapid analysis of light chains from individual animals should also greatly aid such studies.

Summary

Light chains isolated from normal immunoglobulin of unimmunized mice were analyzed by gel isoelectric focusing. Examination of the focusing patterns of light chains from nine inbred mouse strains showed that six of the strains (SWR/J, C3H/HeJ, DBA/1J, A/J, CBA/J, and C57BL/6J) possessed a virtually identical spectrum of focusing bands, while the remaining three strains (RF/J, AKR/J, and C58/J) showed clear differences involving several bands. Analysis of the light chains of individual SWR/J, C58/J, and F_1 hybrid mice indicated that the differences in focusing pattern were inherited in a simple codominant fashion. A new procedure was developed for the rapid analysis of light chains from small quantities of serum.

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