J.L. Claflin

Department of Microbiology, University of Michigan Medical School, Ann Arbor

Genetic marker in the variable region of kappa chains of mouse anti-phosphorylcholine antibodies*

A newly discovered genetic marker in the kappa light chains of mouse immunoglobulins is described. This marker, designated κ-PC8, is located in the L chains of those anti-phosphorylcholine (PC) antibodies which show the same functional and idiotypic characteristics as a PC-binding myeloma protein, HOPC 8 (H8). Analytical isoelectric focusing of these L chains revealed two phenotypes whose strain distribution pattern suggested a genetic association with genes that determine the T lymphocyte surface antigen(s) Ly-2/Ly-3. In four strains, AKR/J, C58/J, RF/J and PL/J (AKR-type, A) the H8-like L chains have a slightly lower isoelectric point than those of C57L/J and 12 other strains (C57L-type, B). Breeding experiments showed that the κ-PC8-A phenotype is preferentially expressed. The most probable location of the marker is the variable region since other idiotypically related κ-chains in C57L/J and AKR/J do not show differences in their electrophoretic mobility.

1. Introduction

Phenotypic markers (idiotypes and fine specificity differences) for at least seven different heavy chain variable (V_H) regions have been described in the mouse. These include the ARS idiotype in the anti-azophenylarsenate response [1], the A5A idiotype in the anti-streptococcal group A response [2], the J558 idiotype in the anti-α-1→3 dextran response [3], the T15 idiotype of the anti-phosphorylcholine (PC) response [4, 5] and three different fine specificity differences described by Mäkelä [6–8]. Studies of the distribution of these markers in inbred strains and their segregation in recombinant mice [9, 10] have shown that the V_H genes coding for these phenotypic markers are arranged in a linear order contiguous to the C_H genes (H chain allotypic markers).

Information on the organization of genes coding for light chain variable (V_L) regions in the mouse is minimal. Ruffilli and Baglioni [11] have identified putative V region antigenic determinants that are shared by mouse and human κ-chains, though the genetics were not analyzed. Edelman and Gottlieb [12] have described a V region marker, I_b, for mouse immunoglobulin L chains. This marker is present in approximately 5 % of the κ-chains and is genetically associated [13] with genes controlling the expression of the Ly-2/Ly-3 alloantigen complex on thymus-derived suppressor lymphocytes [14]. Ly-2/Ly-3 genes have been assigned to chromosome 6 [15]. Identification of genes controlling additional V_L markers and their subsequent mapping are essential to our understanding of a number of elements of the immune response: 1) antibody diversity, 2) various regulatory mechanisms governing proliferation of individual clones of antibody-producing cells and 3) subsequent antibody synthesis by individual cells.

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In this paper we report a newly discovered genetic marker which is located in the κ-chains of one of the species of antibodies that comprise the immune response to PC in mice. The results obtained demonstrate that the marker resides in the V region of κ-chains and is correlated in inbred strains with the expression of I_b and Ly-2/Ly-3.

2. Materials and methods

2.1. Purification of myeloma protein and anti-PC antibodies

The origin and maintenance of the PC-binding plasmacytoma HOPC 8 (H8) has been described in detail elsewhere [5]. All mouse strains were obtained from Jackson Laboratories, Bar Harbor, Maine, with the exception of (AKR/J x C57L/J)F_1 which were raised in our own laboratory. Antisera were pooled from 5–10 mice given two to three intraperitoneal (i.p.) injections of 10^8 heat-killed (56 °C, 30 min) *Streptococcus pneumoniae*, strain R36A [5]. Antibodies specific for PC were isolated from a PC-Sepharose column by affinity chromatography [5]. Isolated mouse anti-PC antibodies were only IgM(κ) by immunoelectrophoresis and by immunodiffusion with class-specific antisera.

2.2. Isoelectric focusing (IEF) of L chains

Purified myeloma proteins and anti-PC antibodies in a volume of one ml containing 300 μg lysozyme (Sigma Chemical Co., St. Louis, Mo.) as carrier protein were first completely reduced with 0.2 M 2-mercaptoethanol in 7 M guanidine in Tris-HCl buffer, pH 8.2 (1 h, 23 °C) and alkylated with 0.4 M iodoacetamide (45 min, 4 °C) [16]. After the reaction, the mixture was dialyzed against phosphate-buffered saline, pH 7.2. The resulting precipitate containing lysozyme, H and L chains was collected by centrifugation and separated into component fractions by disc electrophoresis in sodium dodecyl sulfate according to the procedure of Segrest and Jackson [17]. After electrophoresis the gel was sliced and to the band containing L chains was added 0.02 ml each of Ampholines, pH 5–8 (LKB Instruments, Inc., Rockville, Md.) and 10 % Nonidet P-40. Prior to focusing the gel slice was minced. IEF was performed at 4 °C in a modification of the apparatus described by Reid and Bieleski [18]. The gel was

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Correspondence: J. Latham Claflin, Department of Microbiology, University of Michigan Medical School, 6643 Medical Science Bldg. II, Ann Arbor, Michigan 48109, USA

Abbreviations: H8: HOPC 8 PC: Phosphorylcholine IEF: Isoelectric focusing

composed of 5 % acrylamide (Bio Rad Laboratories, Richmond, Calif.), 2 % ampholytes, pH range 5.0 to 9.5 and 8 M urea (ultrapure grade, Schwarz/Mann Div., Becton, Dickinson and Co., Orangeburg, NY). Samples were focused for 6–7 h at a constant power of 1.5 W. Gels were fixed in a solution of 5 % trichloroacetic acid and 5 % sulfosalicylic acid for 18 h, and stained with Coomassie brilliant blue [19]. Based on studies with isolated H and L chains of mouse IgM antibodies, μ-chains were found to focus in the pH range 5 to 5.8 and L chains in the pH range 4.7 to 8.8 [20].

3. Results and discussion

As shown previously [20], and depicted again in Fig. 1, the L chains of reduced and alkylated myeloma proteins, *e.g.* H8, typically give 4 closely grouped, evenly spaced bands. This microheterogeneity is characteristic of homogeneous myeloma proteins [21], purified antibody preparations [22], and their constituent polypeptide chains [20, 23] and is probably related to pre- and post-synthetic deamidation [21]. In contrast to the restricted pH range of homogeneous L chain bands, L chain banding patterns of anti-PC antibodies are complex and cover the pH range 4.7 to 8.3. However, L chains of immunoglobulin that bears H8 idiotypic determinants focus in a restricted pH range of 7.3 to 8.2 [24]. Thus, as shown in Fig. 1a, the L chains of H8-like anti-PC antibodies from C57L/J and C57BL/6J mice show the same degree of microheterogeneity and migrate to the same positions as H8 L chains. By contrast, L chains of anti-PC antibodies from AKR/J, PL/J and RF/J mice which are idiotypically identical to H8, as well as to C57L/J and C57BL/6J, focus slightly but distinctly, more anodal to them. No additional electrophoretic variants in other strains have been observed. That the two mobility patterns of H8-like L chains are distinct is shown in Fig. 1b. Focusing of L chains from a mixture of C57L/J and AKR/J anti-PC antibodies gave a mixture of two patterns, one identical to the L chains of C57L/J, the other identical to the L chains of AKR/J. The differences in mobility do not appear

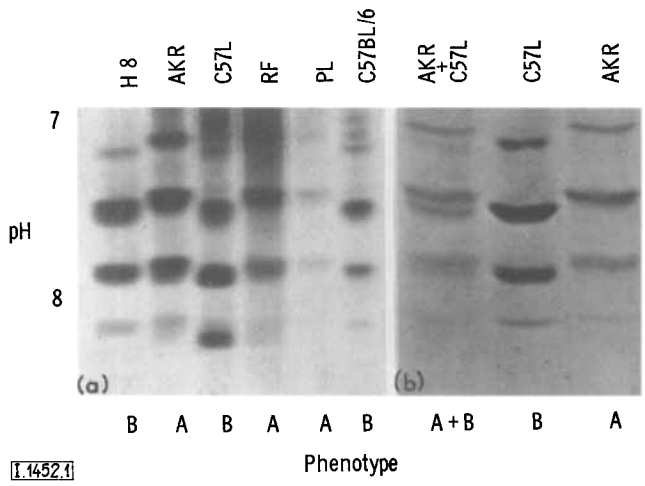

Figure 1. IEF patterns of H8-like L chains. Reduced and alkylated H8 and purified anti-PC antibodies were focused in a thin-layer polyacrylamide gel in pH 5 to 9.5 ampholytes. (a) Demonstration of AKR/J (A) and C57L/J (B) phenotypes in different mouse strains. Sample application at origin was 3 mm in width. (b) Separate samples and a mixture of samples of AKR/J and C57L/J are analyzed. Sample application at origin was 6 mm in width.

to reflect simple deamidation or decarboxylation, since incubation of AKR/J anti-PC antibody in normal C57L/J serum (and *vice versa*) for 18 h at 37 °C did not convert the L chains of AKR into those of C57L/J (and *vice versa*). We will refer to the structural variant causing the differences in mobility of these H8-like L chains as κ-PC8 and recognize two variants, AKR-type or A, and C57L-type or B.

A comparison of the IEF pattern of the L chains of H8-like antibody in a number of different strains is given in Table 1. Of the additional strains tested only C58/J expressed the AKR phenotype; all others expressed the C57L/J patterns. In 8 F_1 progeny of the cross AKR/J x C57L/J only the AKR phenotype was clearly observed. Identical results were obtained with a pool of 5 (C57L/J x AKR/J)F_1 mice and from (AKR/J x DBA/2J)F_1 mice. Extensive overloading with up to 10 times the optimal concentration of the F_1 samples of either cross did not reveal the C57L/J pattern. Whether this dominance is genetic, or represents clonal selection events and immune response gene effects, will have to await analysis of F_2 and backcross mice.

Table 1. Strain distribution of κ-PC8 L chain marker[a)]

Express κ-PC8-A	Express κ-PC8-B
AKR/J, C58/J, RF/J, PL/J (AKR/J x C57L/J)F_1, (AKR/J x DBA/2J)F_1	C57L/J, BALB/cJ, CBA/J, C3H/HeJ, MA/MyJ, ST/6J, 129/J, SEC/ReJ, C57BL/6J, DBA/2J, AL/N, A/J, CE/J

a) κ-PC8 defines an electrophoretic difference in the κ-chains of H8-like antibodies. Males and females of one strain gave the same pattern. Phenotypes A (AKR-type) and B (C57L-type) are defined in Fig. 1. For each strain, anti-PC antibodies isolated from a minimum of two different serum pools were each tested in two separate IEF runs.

Comparison of other sets of related κ-chains in IgM antibody from different mouse strains revealed no differences in pI of the paired bands. In AKR/J (κ-PC8-A) and MA/MyJ (κ-PC8-B), for example, L chains from another anti-PC antibody (Fig. 2) which were idiotypically indistinguishable in the two strains migrated to the same positions; pH 5.6, 6.0 and 6.5 [24]. Identical results were obtained with κ-chains isolated from a third species of anti-PC antibodies found in C58/J and CE/J mice (Fig. 2) which were functionally and idiotypically indistinguishable from each other. These data, though requiring amino acid sequence analysis for confirmation, strongly suggest that the κ-PC8 marker is located in the V region rather than in the Constant region of L chains.

The most striking feature of the strain distribution pattern shown in Table 1 is that it suggests a linkage of κ-PC8 to the I_b peptide marker, a genetic marker found by Edelman and Gottlieb in the neighborhood of the first half-cysteine V region of mouse κ-chains [12]. It is apparent after examination of I_b-positive and I_b-negative strains [13] that all mice possessing κ-PC8-A are also positive for I_b; all other strains are κ-PC8-B and I_b-negative. Thus, it appears that genes controlling the expression of κ-chains are located on chromosome 6 in the mouse. Although it was conceivable that κ-PC8 may be the I_b peptide, comparison of the sequence of I_b and H8

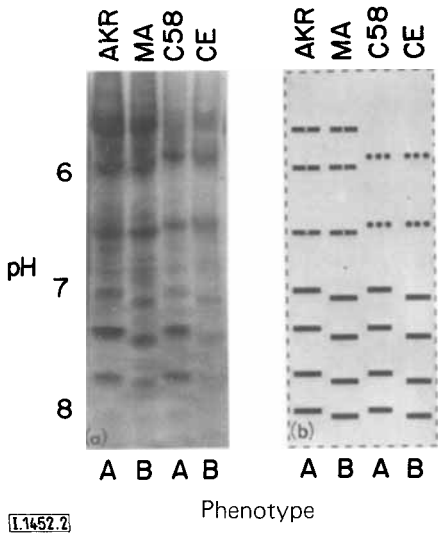

Figure 2. (a) IEF patterns of mouse L chains. (b) Diagrammatic representation of (a). Reduced and alkylated L chains from anti-PC antibodies were focused as described in Fig. 1. Bands migrating at pH 7.3–8.2 (—) are derived from H8-like anti-PC antibodies. Bands migrating at pH 5.9 and 6.4 in CE/J and C58/J mice (· · ·) are κ-chains from a second species of anti-PC antibodies which are homogeneous and idiotypically indistinguishable in the two strains. Bands at pH 5.6, 6.0 and 6.5 in AKR/J and MA/J mice (– –) represent κ-chains from a third species of idiotypically identical anti-PC antibodies [24].

argues against this. In the critical region of half-cysteine I in the V_L region (position 19–24), H8 (and probably κ-PC8-B) L chains show the sequence Val-Thre-Ile-Ser-Cys-**Thre** [25]. A comparable sequence for AKR/J H8-like chains based on amino acid sequence analysis of AKR/J I_b [12] would be Val-Thre-Ile-Ser-Cys-**Lys**. This substitution of lysine for threonine would increase the positivity of AKR/J H8-like L chains, causing these to migrate more cathodal than H8 L chains. Since AKR/J L chains actually migrate more anodal than C57L/J L chains, I_b cannot represent κ-PC8. As a consequence, κ-PC8 appears to be an additional independent marker for κ-chains in the mouse. Structural studies now in progress using H8 and AKR/J anti-PC H8-like L chains should identify the difference(s) as well as determine the markers' relationship to I_b. In addition, antisera which recognize this genetic polymorphism should provide information on the distribution of the κ-PC8 marker in other κ-chains.

Genetic analysis for linkage of genes controlling the expression of κ-PC8-A are in progress. In preliminary experiments with Taylor's AKXL mice (recombinant inbred lines derived from (AKR/J x C57L/J)F_2 [26], κ-PC8 appears to be closely linked to Ly-2/Ly-3 (Claflin and Taylor, unpublished data). If current studies confirm linkage of κ-PC8 to Ly-2/Ly-3, analysis of the number and organization of V_L genes as well as their relationship to Ly-2/Ly-3 becomes possible.

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