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Major urinary protein and immunoglobulin allotypes of recombinant inbred mouse strains

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SUMMARY

Serum and urine samples from seven recombinant inbred mouse strains, derived from a cross between BALB/c and C57BL/6, were examined to determine the immunoglobulin heavy chain (IgC_H) and the major urinary protein (MUP) allotypes. CXBG and CXBJ exhibited the same IgC_H alleles as did BALB/c; the others resembled C57BL/6, thus providing no evidence of crossover types. Comparison of the *Mup* and brown coat colour (*b*) alleles (both on linkage group VIII) revealed that three of the strains resemble BALB/c and two resemble C57BL/6, whereas the CXBE and CXBI strains are crossover types.

1. INTRODUCTION

Seven new inbred strains of mice, derived from a cross between BALB/c and C57BL/6 and subsequently maintained by sib mating (Bailey, 1971), are coming into wide use in the fields of immunology and biochemical genetics. The strain distribution pattern of alleles at three coat colour loci and eight histocompatibility loci has been described (Bailey, 1971). We report here the immunoglobulin IgC_H allotypes (Potter & Lieberman, 1967; Minna, Iverson & Herzenberg, 1967) and the major urinary protein (MUP) electrophoretic allotypes (Finlayson, Potter & Runner, 1963; Hudson, Finlayson & Potter, 1967) exhibited by these strains.

2. METHODS

Allotypes of three of the immunoglobulin classes were determined by a micro-Ouchterlony immunodiffusion method with four different allotypic antisera, each of which distinguished a characteristic, non-crossreacting determinant on BALB/c or C57BL immunoglobulins. (1) Antiserum to the BALB/c HOPC1 myeloma protein (Potter, 1972) was prepared in strain LP mice; it specifically identifies immunoglobulins bearing the $G^{1,6,7,8}$ (Ig1.10; see Herzenberg, McDevitt & Herzenberg, 1968) determinants found on BALB/c immunoglobulins. (2) Antiserum to normal C57BL/Ka immunoglobulin, prepared in BALB/c mice, is specific for the unassigned 2 (Ig1.4) determinant found on C57BL immunoglobulins. (3) Antiserum to MOPC467 γA myeloma protein, prepared in A/J mice, is specific for the A^{12} (Ig2.2) determinant on BALB/c γA immunoglobulins. (4) Antiserum to MOPC352 myeloma protein, prepared in YBR mice, is specific for the H^{16} (Ig3.9) determinant on C57BL immunoglobulins. The MOPC352 plasmacytoma was induced in a BALB/c·C57BL/Ka IgC_H congenic mouse; the constant

portion of the heavy chain of this protein is controlled by the C57BL/Ka IgC_H locus (Potter & Lieberman, 1967). The γF (γ1) allotypes were determined by the immunoelectrophoretic method of Minna *et al.* (1967). Whole serum was digested with papain, electrophoresed in agar gel, and allowed to react with a rabbit anti-mouse serum specific for γF (γ1) immunoglobulins.

Urine samples were collected, dialysed, and freeze-dried, and the non-dialysable fraction was dissolved, all by procedures which have been described previously (Hudson *et al.* 1967; Finlayson, Hudson & Armstrong, 1969). Agar–gel electrophoresis was carried out at pH 5·5 with tris(hydroxymethyl)aminomethane acetate buffer, ionic strength 0·05 (Finlayson *et al.* 1963). Determinations of MUP types were confirmed by cellulose acetate electrophoresis, which was performed with this same buffer in a Beckman Microzone ® cell.

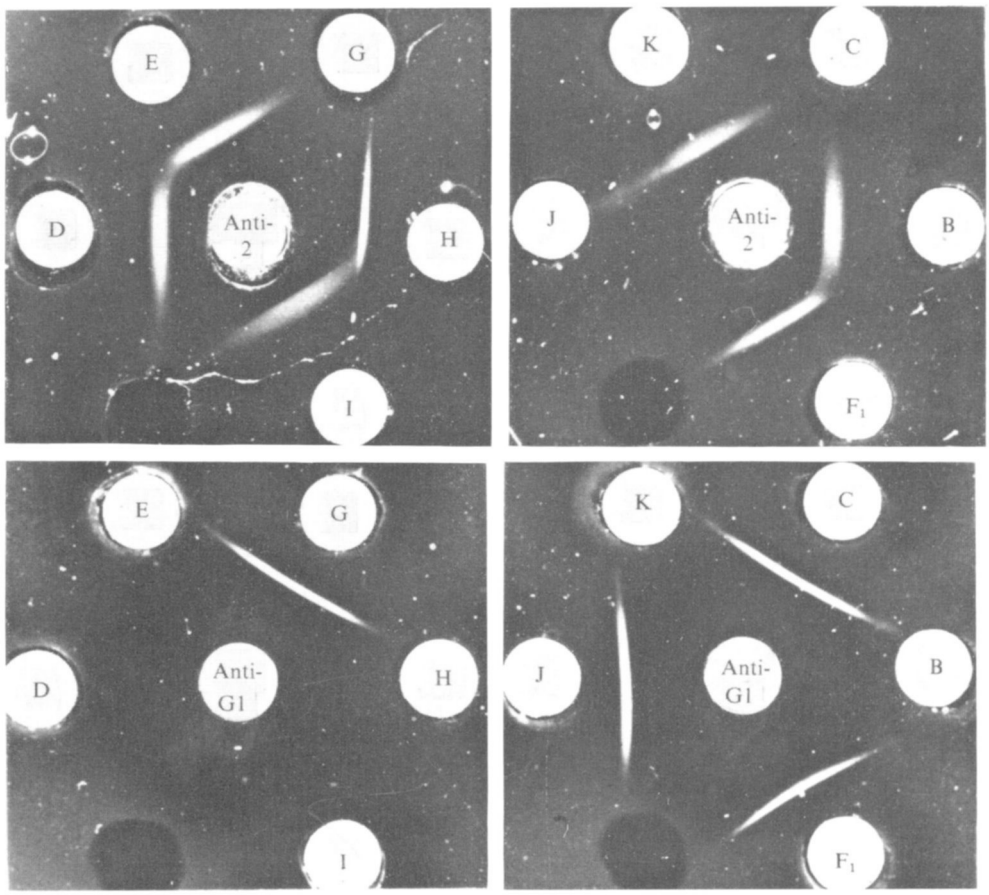
3. RESULTS AND DISCUSSION

Immunodiffusion experiments with normal sera from BALB/c, C57BL/6, C57BL/Ka, and the seven recombinant inbred strains demonstrated that the sera from BALB/c, CXBG, and CXBJ mice all precipitated with the LP anti HOPC1 (lower portion, Plate 1) and the A/J anti MOPC467 (not shown) sera. On the other hand, sera from C57BL and the remaining five recombinant inbred strains precipitated with the BALB/c anti C57BL/Ka serum (upper portion, Plate 1) and with the YBR anti MOPC352 serum. Serum from the (BALB/c × C57BL/Ka) F_1 hybrid precipitated with all four antisera.

Portions of all sera were digested with papain to release the γF (γ1) Fc fragment and examined by immunoelectrophoresis with the rabbit antiserum. The BALB/c, CXBG and CXBJ sera exhibited the fast (F) type fragment, whereas C57BL serum and the sera from the other recombinant inbred strains had the slow (S) type. These results, summarized in Table 1, indicate that CXBG, CXBJ and BALB/c mice have the same alleles at the IgC_H locus and provide no evidence of crossover types among the seven recombinant inbred strains.

Typical electrophoretic patterns of the MUP complex are illustrated in Plate 2. MUP component 1 (the characteristic MUP component in BALB/c) was present in CXBE, CXBG, CXBH and CXBJ. The other three strains exhibited component 2, as do C57BL mice. [Component 3 has been observed in the urine of all strains tested, though the amount relative to component 1 or 2 varies with the strain and sex (Finlayson *et al.* 1963).] In Table 1 these results are summarized and compared with the alleles present at the brown (*b*) coat colour locus. The *b* and *Mup* loci both occur on linkage group VIII (Hudson *et al.* 1967), i.e. chromosome 4, and are located approximately 5 map units apart (Finlayson *et al.* 1969). On this basis, comparison of the allelic patterns with those of BALB/c and C57BL/6 revealed that two strains, CXBE and CXBI, are crossover types.

Prior to the present study the MUP types of more than 60 strains and sublines had been determined (Finlayson *et al.* 1963; Finlayson & Potter, unpublished data; Hoffman, 1970; Hudson *et al.* 1967; Reuter *et al.* 1968; Roberts, unpublished data). In 50 of these the allele at the *b* locus was also known. When the latter group was examined statistically, *Mup-a*^{1} (*Mup-1*^{a}) was found to be linked in coupling with *b* in 20 cases and *Mup-a*^{2} (*Mup-1*^{b}), in coupling with *B* in 13 – a distribution which appeared to differ significantly from that predicted by the null hypothesis ($P < 0{\cdot}02$). However, when individual substrains were not considered separately (e.g. all C57BL substrains treated as a single strain), the relative frequencies of the coupling and repulsion combinations of these alleles did not differ significantly (Table 2). Moreover, both reciprocal crossover types were also found in the recombinant inbred strains tested in the present study. This, of course, is as expected if there is no survival advantage to one combination or the other. On the other hand, the significantly greater frequency of the *b* and *Mup-a*^{1} (*Mup-1*^{a})



Immunodiffusion of serum from inbred and hybrid mice. Abbreviations: D, CXBD; E, CXBE; G, CXBG; H, CXBH; I, CXBI; J, CXBJ; K, CXBK; B, C57BL; C, BALB/c; F_1, CXB F_1 hybrid; anti G1, LP anti HOPC1 serum specific for the $G^{1,6,7,8}$ determinants on BALB/c immunoglobulins; anti 2, BALB/c anti C57BL/Ka serum specific for the unassigned 2 determinant on C57BL immunoglobulins. Sera from both C57BL/6 and C57BL/Ka mice were tested, and identical results were obtained. The serum illustrated here was from C57BL/Ka mice.

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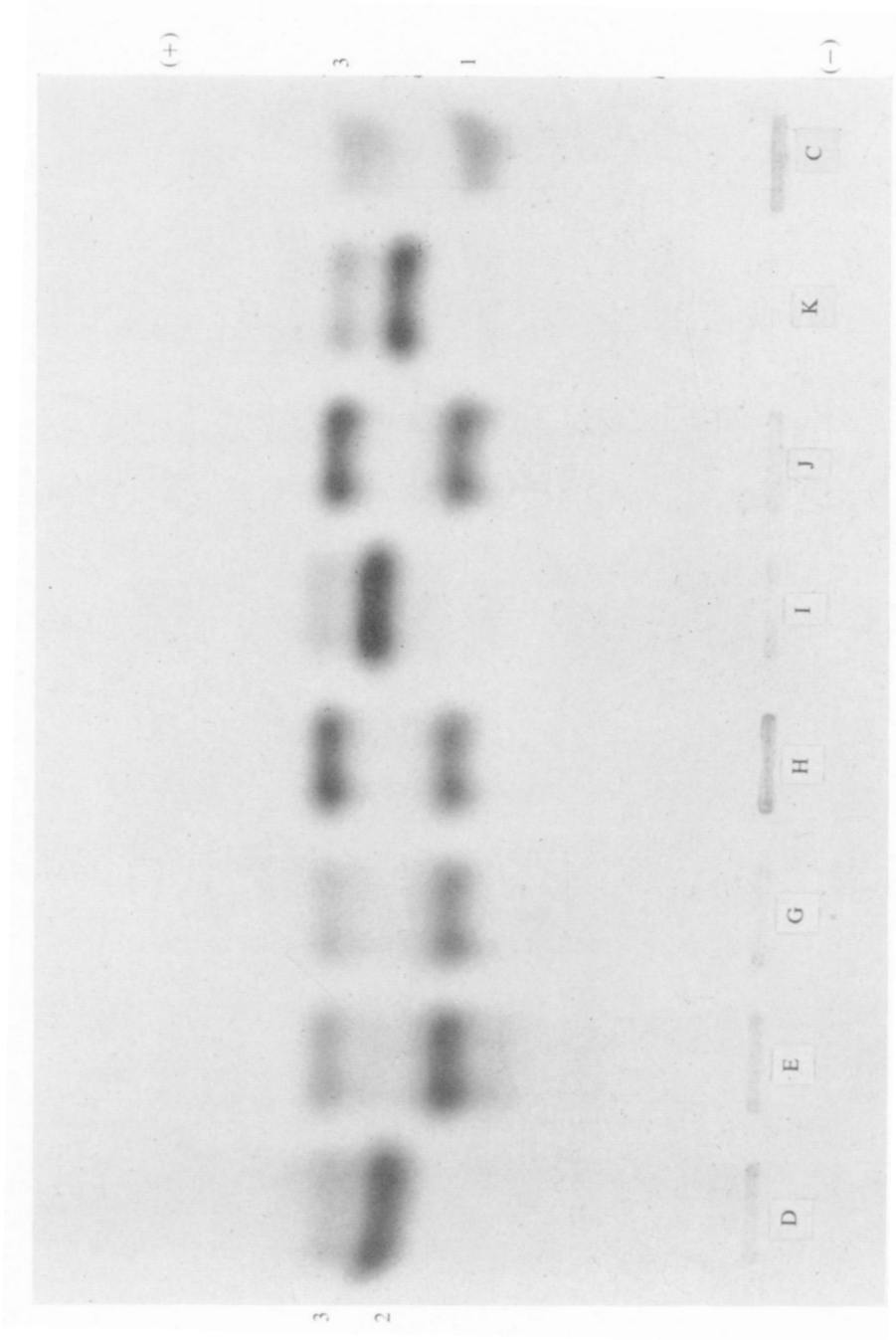

Cellulose acetate electrophoresis of urinary proteins from inbred mice. Abbreviations: D, CXBD; E, CXBE; G, CXBG; H, CXBH; I, CXBI; J, CXBJ; K, CXBK; C, BALB/*c*. Electrophoresis was carried out for 30 min at a potential of 250 V; staining was done with Ponceau S. Samples shown were from female mice.

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Table 1. *Comparison of* b *alleles with MUP and immunoglobulin phenotypes of recombinant inbred strains*

Strain	*b* allele*	Electrophoretic MUP components	Electrophoretic γF (γ1) Fc type	Allotypic antigenic determinant†			
				2(Ig1.4)	G^1(Ig1.10)	H^{16}(Ig3.9)	A^{12}(Ig2.2)
CXBD	*B*	2, 3‡	Slow	+	−	+	−
CXBE	*B*	1, 3	Slow	+	−	+	−
CXBG	*b*	1, 3	Fast	−	+	−	+
CXBH	*b*	1, 3	Slow	+	−	+	−
CXBI	*b*	2, 3	Slow	+	−	+	−
CXBJ	*b*	1, 3	Fast	−	+	−	+
CXBK	*B*	2, 3	Slow	+	−	+	−
BALB/c	*b*	1, 3	Fast	−	+	−	+
C57BL	*B*	2, 3	Slow	+	−	+	−

* Determined by Bailey (1971).

† Determined by micro-Ouchterlony immunodiffusion. See text for description of the antiserum used in detecting each determinant. The designations given in parentheses are those employed in the Herzenberg nomenclature system (Herzenberg *et al.* 1968).

‡ On starch-gel electrophoresis at pH 9·1, the MUP components 1 and 3 detected by agar gel electrophoresis at pH 5·5 appear as three bands, whereas components 2 and 3 resolve into four bands (Hoffman, 1970).

Table 2. *Distribution of* b *and* Mup *alleles in major strains**

b allele	*Mup* allele	Strains
b	*Mup-a*^{1} (*Mup-1*^{a})	A, AL, BALB/c, BL, BRSUNT, C57BR, DBA, F, I, N, PBR, ST, STR
B	*Mup-a*^{1} (*Mup-1*^{a})	AKR, CBA, CE, C3H, DD, Flexed, NBL, WB†
b	*Mup-a*^{2} (*Mup-1*^{b})	C57L, HR, P, POLY, YBR
B	*Mup-a*^{2} (*Mup-1*^{b})	C57BL, C58

* The seven recombinant strains listed in Table 1 are not repeated here. 'Major' strains implies no discrimination among substrains and sublines; e.g. DBA includes several lines of DBA/1 and DBA/2, C57BL includes all lines of this strain examined, etc.

† WC also carries these alleles.

alleles at their respective loci (Table 2) indicates either that they have been at a selective advantage or, as is more likely, that they were more frequent in the gene pool of the original laboratory stocks of mice.

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