Genet. Res., Camb. (1975), **26**, *pp.* 213–219
With 1 text-figure
Printed in Great Britain

Inheritance of the macrophage alloantigenic marker (Mph-1) in inbred mice

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(*Received* 20 *August* 1975)

SUMMARY

Improvements in cytotoxic technique allowed a determination to be made of the strain distribution and inheritance of Mph-1, the mouse peritoneal exudate cell alloantigen. *Mph-1*^{b}, the allele in B10 lines, is inherited as a Mendelian co-dominant gene. In a 3-point backcross using the colour markers pink eye dilution (*p*) and chinchilla (c^{ch}) it was shown to map about 36 units from *p* in the order *p*–c^{ch}–*Mph-1* on chromosome 7. An alternative antigen, Mph-1.1, has been identified on strains F/St and I/StDa.

1. INTRODUCTION

The mouse alloantigen Mph-1.2 is identified by a cytotoxic reaction on a subpopulation of unstimulated peritoneal exudate cells (Archer & Davies, 1974). It was first detected by a reaction of an I/StDa anti-B10.M serum and occurs in most strains of inbred mice. Originally we reported indirect evidence that the antigen was coded on chromosome 7 (linkage group I), but the gene could not be mapped more closely because of the erratic results obtained using target cells from heterozygous mice. This paper describes improvements in the technique for determining alloantigens of peritoneal exudate cells and results relating to the inheritance and expression of *Mph-1*.

Whilst mapping *Mph-1*^{b} we found that our line of I strain mice (I/StDa) carried a different set of colour genes from the ones we had expected (Staats, 1972), and colour typing of I/StDa is also briefly described.

2. MATERIALS AND METHODS

(i) *Mice*

Strains A/J, C57BL/6, C57BL/6-Tla, C57BL/10, 129, B10.D2/n, BALB/c, DBA/2, B10.M, I/StDa, B10.BR, A.SW, NZB, NZW and all hybrid mice except where otherwise stated were bred in the SPF unit of Searle Research Laboratories, High Wycombe. C57L and F/St were obtained from the M.R.C. Laboratory Animal Centre, Carshalton, Surrey, and SW.R and C57BL/6-A$^{y}c^{ch}$ mice from the Jackson Laboratory, Bar Harbor, Maine.

(ii) *Skins*

Where they were needed for comparison, the skins were lightly stuffed with cotton wool to maintain a rounded shape, and freeze-dried for 2–3 days. They were stored in the dark in plastic bags with paradichlorobenzene crystals.

(iii) *Peritoneal exudate cells*

Shiga-stimulated cells (SPEC) for immunization, and unstimulated cells (NPEC) for targets were obtained as previously described (Archer & Davies, 1974). Except where otherwise stated all manipulations were carried out in Eagle's Minimal Essential Medium buffered with HEPES and containing foetal calf serum (10%) (HEPES-MEM).

(iv) *Antisera*

(*a*) *S127* (*Anti-Mph-1.2*). The preparation of this serum has been described (Archer & Davies, 1974): briefly I/StDa × (B10.M × I/StDa) mice typed as heterozygous for $H\text{-}2^t$ and $H\text{-}2^j$ were immunized with B10.M SPEC, and the active antisera pooled.

S140 (*Anti-Mph-1.2*). SPEC from B10.M were injected weekly for 6 weeks at a level of 5×10^7 cells/mouse into recipients which were heterozygous for $H\text{-}2^t$ and $H\text{-}2^j$ (from B10.M and I/StDa respectively) and homozygous for $Mph\text{-}1^a$ (from I/StDa). Mice were bled 14 days after the last injection.

S141 (*Anti-Mph-1.1*). I/StDa SPEC were injected into 10 mice (originating from progeny of matings between I/StDa × (B10.M × I/StDa) backcross mice) which were selected as homozygotes for the I/StDa $H\text{-}2^j$ allele and some of which were expected to be homozygous for the B10.M $Mph\text{-}1^b$ allele. The injection schedule was similar to that for S140. Active antiserum was obtained from 1 mouse.

(v) *Cytotoxicity tests*

These were carried out by a modification of the methods of Sanderson (1965) and Wizgell (1965). NPEC collected in HEPES-MEM in ice-cold siliconized glass tubes were centrifuged once at 1100 *g* (5 min) and then incubated with sodium chromate (Radiochemical Centre, Amersham, Bucks) at a level of approximately 400 μCi/2×10^6 cells for 60 min at room temperature (20 °C). They were then washed by centrifugation 5 times in ice-cold medium and resuspended at 1×10^6 cells/ml in HEPES-MEM containing sodium azide (0·1 mg/ml). Doubling dilutions of antiserum were made in 25 μl aliquots in plastic conical welled Cooke microtiter trays (Dynatech Laboratories Ltd, Billingshurst, Sussex) in HEPES-MEM with azide, and after addition of cells (25 μl) and suitably diluted guinea-pig complement (25 μl) the trays were incubated at a minimum temperature of 25 °C for 90 min. The reaction was stopped with EDTA and the amount of chromium released measured by standard techniques. The use of azide gave more consistent

results, presumably because it prevented phagocytosis of antibody and complement by macrophages. If cells were kept in it for more than 90 min, or the test was run at 37 °C, azide caused an unacceptable rise in background.

3. RESULTS

(i) *Mendelian inheritance of* Mph-1^b *and linkage to pink eye dilution* (p)

In an initial series of experiments 63 mice of the backcross I/StDa × (B10.M × I/StDa) were typed for colour and for Mph-1.2 using serum S127. Some reaction with this antiserum could be detected with most of these mice but the titres were rather variable, suggesting that more than one peritoneal cell alloantigen was

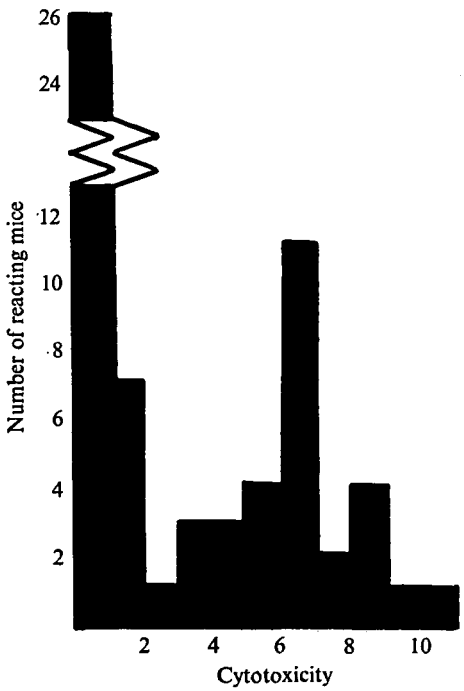

Fig. 1. Complement-dependent cytotoxicity of anti-Mph-1.2 serum (S127) against unstimulated peritoneal exudate cells from I/StDa × (B10.M × I/StDa) backcross mice. 'Cytotoxicity' is proportional to the ^{51}Cr-release titration curve.

segregating. When the titres were plotted on semilog graph paper, and 'integrated titres' found by weighing the paper lying between the titration curve and the complement control, it became clear that the mice fell readily into two groups (Fig. 1), 33 giving a major cytotoxic reaction (2 units and over) and 30 giving a minor reaction. The major reaction was taken to represent Mph-1.2 positive heterozygotes and the minor reaction Mph-1.1 homozygotes. The pattern is consistent with inheritance of a Mendelian dominant or co-dominant gene from B10.M.

https://doi.org/10.1017/S0016672300016001 Published online by Cambridge University Press

I-strain mice are listed as carrying the genes *a* (*non-agouti*), *b* (*brown*), *d* (*dilute*). *ln* (*leaden*), *p* (*pink-eye dilution*) and *s* (*piebald*) (Staats, 1972). The incidence of *Mph-1*^{b} was compared with *p*, *s*, *b*, *d* (or *ln*) and the sex of the backcross mice. There was no evidence of an association of *b* or *s* with *Mph-1*, or of sex linkage. However, an association was found with *p* which was significant at the 1% level (Table 1), confirming that the *Mph-1* gene is carried in chromosome 7. Linkage estimates made the distance of *Mph-1* from *p* to be 31·7 ± 5·9 centimorgans.

Table 1. *Association of Mph-1 with p in an I/StDa × (B10.M × I/StDa) backcross using serum S127*

	P	*p*	Linkage 31·7 ± 5·9
Mph-1.2 positive	20	10	$\chi^2_1 = 8{\cdot}4$, $0{\cdot}001 < P < 0{\cdot}01$
Mph-1.2 negative	10	23	

Table 2. *Determination of I/StDa colour genes*

Parent (with I/StDa)	Relevant parental colour genes	Phenotype or description of offspring	I/StDa genotype
DBA/2	*aabbdd*	*abd*	*aabbdd*
C57L	*aabblnln*	*abLn*	*aabbLnLn*
A/J	*aabbP-c/P-c*	Light brown, black eye	*aabbc*?
BALB/c	*AABBP-c/P-c*	Very light agouti, black eye	*c*?
129	$A^wA^wp\text{-}c^{ch}/p\text{-}c^{ch}$	$A^wp\text{-}c^{ch}$	$p\text{-}c^{ch}/p\text{-}c^{ch}$?
129 × (B10.D2 × 129) (black-eyed chinchilla)	$A^w?P\text{-}c^{ch}/p\text{-}c^{ch}$	$A^wp\text{-}c^{ch}$ and $A^wP\text{-}c^{ch}$	$p\text{-}c^{ch}/p\text{-}c^{ch}$?
C57BL/6-A^{y}c^{ch}	$A^yaBBP\text{-}c^{ch}/P\text{-}c^{ch}$	$aBP\text{-}c^{ch}$ and Black eyed, darker than parent A^yc^{ch} but not yellow. Ears very pale, belly white. Back variable, pale buff or brownish-grey, changes on moulting	Probably $c^{ch}c^{ch}$

(ii) *Colour genes in I/StDa mice*

The genes *d* and *ln* are not linked to *p*, but an early impression was obtained that one of them was linked to *Mph-1*. It soon became apparent that this was due to an error in identifying pink-eyed very pale mice, and we decided to check the colour genes of our own I/StDa strain using standard strains. The results of these matings are shown on Table 2. They show that I/StDa carried the genes *p*, *d* and *b* but not *ln*. There is, however, another diluting gene linked to *p* which is identical or very similar to *c*^{ch} (*chinchilla*).

(iii) *Position of* Mph-1 *relative to* c^{ch} *and* p

The presence of two colour markers *p* and *c*^{ch} on the same chromosome allowed us to carry out a three-point cross using the I/StDa × (B10.M × I/StDa) backcross. Serum S140 was used to test 118 backcross mice for presence of Mph-1.2. The skin from each mouse was freeze-dried, and when the cytotoxicity testing was com-

pleted, all of the skins were compared, and as far as possible, assigned genotypes. Phenotype *p* is easily assigned, and amongst black-eyed mice so are *d*, *b* and *s*. Wild-type *P* mice homozygous for c^{ch} have white hairs in their ears and can readily be identified in the *PDBS* and *PDbS* phenotypes. We did not, however, attempt to identify c^{ch} in *Pd* mice, or in *Ps* if the white patches came near the ears. This left only 14 black-eyed (*P*) to which the gene c^{ch} could be positively assigned. Mice homozygous for *p*-c^{ch} are much paler than *p*-*C* regardless of whether genes from the *d* or *b* loci are present. Crossovers could therefore be readily identified amongst all the pink-eyed mice. The *p*-c^{ch} mice were very pale, and showed a gradation in fur colour from obvious blackish grey to white, so it was not always possible to be confident about which genes a mouse carried from the loci *b*, *d* and *s*. The attempt

Table 3. *Association of Mph-1 with other phenotypes in an I/StDa × (B10.M × I/StDa) backcross using serum S140*

	P	*p*	*PC*	*Pc*^{ch}	*pC*	*pc*^{ch}
Mph-1.2 positive	43	25	12	0	11	14
Mph-1.2 negative	20	30	0	2	1	29
Linkage	38·2		21·8			
χ_1^2	6·64		—			
P	0·01		—			

was nevertheless made as a check on the previous conclusion that none of these genes outside the 7th chromosome did not differ significantly from 50%. Table 3 shows the associations found within the chromosome for all 118 mice. Amongst the black eyed mice 2/14 (14%) were crossovers between *p* and c^{ch} and both were also crossovers between *p* and *Mph-1*. Amongst pink-eyed mice 12/55 (22%) were crossovers between *p* and c^{ch}, but only 1 of these crossovers (9%) was not also a crossover with *Mph-1*. It was concluded therefore that the genes are in the order *p*–c^{ch}–*Mph-1*.

Combination of the data in Tables 1 and 3 gives a crossover distance of 35·9 ± 3·6 centimorgans. However, the second set of data gives a ratio of homozygotes to heterozygotes which is suspiciously lower than 1 ($0{\cdot}05 < P < 0{\cdot}1$) and the estimate may be high.

(iv) *Strain distribution of Mph-1.1 and Mph-1.2*

The strain distribution of Mph-1.2 published in our previous paper was checked using serum S140 and the azide cytotoxic technique, and is summarized in Table 4. The only significant difference was that B10.D2/n reacted positively, a result originally expected because it is derived from two strains C57BL/10ScSn and DBA/2 which are both positive. Strain F/St gave a negative reaction.

When putative anti-Mph-1.1 serum S141 (which, like anti-Mph-1.2, reacted with NPEC but not lymph node cells) was reacted with the same panel, F/St, I/StDa and

(B10.M × I/StDa) F_1 mice were strongly positive and A.SW and AKR gave weak reactions. We do not know whether weak reactions represent more specificities in the serum (which is not congenically derived) or if the *Mph-1* locus is more complex than we originally hoped. The results with I/StDa, F/St and the (B10.M × I/StDa) F_1 show that the two original Strong lines both express the alternative allele, and that *Mph-1*^{a} and *Mph-1*^{b} behave as Mendelian codominants.

Table 4. *Strain distribution of Mph-1.1 and Mph-1.2*

Mph-1.1 (*Mph-1*^{a})	F/St, I/StDa
Mph-1.2 (*Mph-1*^{b})	A/J, (A.SW), C57BL/10ScSn, (B10.A, B10D2/n, B10.M, B10.BR), C57BL/6-*T1a*, 129, BALB/c, DBA/2, NZB, C3H/He, CBA/Ca, AKR, DBA/1, SW.R, NZW.

4. DISCUSSION

The mapping data confirm that Mph-1 is a new antigen. An abbreviated summary of chromosome 7 linkages, including histocompatibility antigens, is

$$Gv\text{-}2 \ldots 18\ \% \ldots \underline{Mph\text{-}1 - 2\ \% - fr} - 3\ \% - ol - 9\ \% - \underline{H\text{-}1 - 2\ \% - Hbb} - 6 - c - 14\ \% - p,\ H\text{-}4 - 16\ \% - H\text{-}22 - ,$$

where brackets indicate that the order in which a pair maps is uncertain (*Gv-2* is Gross virus 2, *fr* is frizzy, *ol* is oligodactyly, *H-1*, *H-4* and *H-22* are histocompatibility antigens and *Hbb* is haemoglobulin β-chain (Green, 1974)).

Co-dominance of alloantigenic markers, as with polymorphic biochemical markers, appears to be the rule rather than the exception, and in this respect Mph-1 behaves like H-2 and Thy-1. It is interesting that both Mph-1.1 positive strains of mice originated in Strong's laboratory and carry the linked c^{ch} gene. Presumably they inherited the *Mph-1–*c^{ch} stretch of chromosome from the same ancestor.

It is not obvious in the experiment summarized in Table 3 why we failed to obtain equal numbers of homozygotes and heterozygotes. Adult I/StDa mice have peritoneal macrophages perfectly capable of phagocytosing carbon (unpublished data). We had previously noticed deficiencies of mice homozygous for colour genes linked to *Mph-1*^{a} in batches produced outside our main breeding unit and confirmed that this was not due to losses after birth.

There is no direct evidence to explain why sodium azide should help to give more consistent results in the cytotoxicity text. However, respiratory poisons are known to inhibit pinocytosis (Cohn, 1966) and capping (Taylor *et al.* 1971) and the most likely explanation is that it prevents removal of antibody and/or complement from the cell surface before they have a chance to act. We have never found any need for the assistance of a metabolic inhibitor in cytotoxicity tests using lymphocytes and thymocytes but the device may be useful in tests involving actively phagocytosing cells, such as macrophages or polymorphonuclear leucocytes.

I would like to thank Mrs Daphne Smith for her skilful technical assistance.

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