Mast cells in spotted mutant mice (\(W Ph mi\))

By J. Stevens† and J. F. Loutit, F.R.S.

M.R.C. Radiobiology Unit, Harwell, Didcot OX11 0RD, U.K.

(Received 15 March 1982)

Mast cells derived from haematopoietic tissue are deficient in numbers in spleen, stomach and skin of Harwell mice doubly mutant at the spotting \(W\) locus: seven viable combinations of four mutants. Most combinations have variably impaired viability, anaemia and infertility; but homozygous \(W^{sh} W^{eh}\) are normal in these respects yet still lack mast cells. The effect of the \(W\) gene on mast cells acts in recessive fashion. Effects of doubly mutant \(W\) genes on mast cells and coat colour, the latter usually regarded as dominant, appear more closely related than other pleiotropic effects. The spotting gene \(Ph\), closely linked to \(W\), has but marginal effects on mast cells, whereas \(mi\), another spotting gene, quite unrelated to \(W\) affects mast cells in the spleen in a dominant way. Thus, splenic mast cells may be a special category of a heterogeneous population.

Peptic ulceration, recorded in \(W/W^v\) mice of Jackson stock, was not seen in Harwell mice. We suggest that this lesion is due to genetic complementation or environmental causes.

Introduction

Mast cells, once thought to be cells intrinsic to connective tissue, are derived from immigrant precursors arising in haematopoietic tissue (Kitamura et al. 1977). Many recent studies have involved marked haematopoietic cells given to mast cell deficient \(W/W^v\) mice (Kitamura et al. 1978, 1981).

Furthermore it was reported that peptic ulceration was common in \(W/W^v\) mice of Jackson stock bred and maintained in Osaka (Shimada et al. 1980). No such ulceration had been noted in Harwell stock of doubly mutant \(W\) mice. It has since been learnt (Kitamura et al. 1980) that Jackson stock on arrival in Osaka exhibit few ulcers, so that environmental causes may be involved.

Therefore, Harwell stock of \(W\) mutant mice were examined for mast cells in tissues and for peptic ulceration. This stock also included the \(Ph\) gene (a spotting gene closely linked with \(W\) on chromosome 5). In the same room, sharing the environment, was stock containing \(mi\), another spotting gene on chromosome 6. Their tissues were likewise examined.

† Present address: South African Institute for Medical Research, Port Elizabeth, South Africa.
J. Stevens and J. F. Loutit

**Materials and methods**

**Mice**

Harwell stock included four mutants: $W^v$ (Little & Cloudman 1937), of long standing; and three others identified locally, $W^e$ (Cattanach 1978), $W^{sh}$ (Lyon & Glenister 1978) and $W^{19H}$ (unreported). Each is an outbred stock derived from C3H/HeH $\times$ 101/H. $W^v$ and $W^{sh}$ are viable as homozygotes, $W^e$ and $W^{19H}$ are not, but may form viable compounds with $W^v$ and $W^{sh}$. The same hybrid stock contained $Ph$.

Bone marrow from this stock was in use to rescue lethally irradiated $mi/mi$ osteopetrotic mice from the radiation syndrome and as a consequence to cure osteopetrosis (Loutit & Nisbet 1979, 1982). The $mi/mi$ were either from strain G mice of the laboratory or from hybrids of this strain and CBA ($m_i$) into which $mi$ had been repeatedly backcrossed (Loutit & Sansom 1976). All mice were bred and used experimentally in the same room, therefore with common environments.

**Mast cells in tissues**

Mice of the stocks above were killed and their tissues, skin, spleen and ‘stomach’ (containing abdominal oesophagus and proximal duodenum) opened along the greater curvature, were fixed in formol acetic acid according to the method of Enerbäck (1966) since it was there reported that this fixation is required to reveal mast cells in the gut of rats. The tissues embedded in paraffin were cut at 3 μm and stained with toluidine blue, pH 4. Mast cells were counted in dermis, submucosa of stomach (of which the various parts, oesophagus, forestomach, glandular stomach and duodenum, were not at variance) and the whole area of spleen by one observer using an eyepiece graticule of known grid size. The results were calculated as numbers of mast cells per square micrometre, so that the results for different tissues should be comparable.

**Statistics**

Means and their standard errors were calculated by standard methods but the numerical comparisons were done with non-parametric tests: the Mann–Whitney for two sample comparisons; the Kruskal–Wallis for more than two comparisons.

**Results**

Table 1 records the results in mice containing zero, one and two $W$ mutants in their genome. Mice doubly mutant at $W$ in all seven viable combinations were notably deficient in mast cells, in all three tissues as reported previously for $W/W^v$, $F_1$ mice of two inbred stocks. The $W^v/W^v$ homozygote was, in two out of three instances, notably in skin, rather less deficient than the others (cf. Go et al. 1980). The spleen was consistently void of mast cells in all. In heterozygotes the figures for gut were significantly lower and those for spleen higher than in non-mutants, but not significantly so.

The linked gene $Ph$ (Grüneberg & Truslove 1960), which is lethal in double dose,
had only minor effects as heterozygote on mast cells but as double heterozygote with a mutant \( W \) gene gave some exaggeration in values in gut (table 1, (5)).

The unlinked spotting gene \( m^i \) (Hertwig 1942) was notable in causing absence of mast cells in spleen both as heterozygote and homozygote (table 2). It was without effects as heterozygote in gut and skin and as homozygote in skin, though

### Table 1. Number of Mast Cells per Square Micrometre in Three Tissues
of Mice of Five Genotypes

(Mean ± s.e.)

<table>
<thead>
<tr>
<th>genotype</th>
<th>(a) gut</th>
<th>(b) skin</th>
<th>(c) spleen</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) +/+</td>
<td>56.4 ± 6.1</td>
<td>72 ± 29</td>
<td>13.9 ± 1.5</td>
<td>0.014</td>
</tr>
<tr>
<td>(2) ( W^*/ + )</td>
<td>28.4 ± 2.5</td>
<td>68 ± 23</td>
<td>34.8 ± 9.3</td>
<td>0.38</td>
</tr>
<tr>
<td>(3) ( W^<em>/ W^</em> )</td>
<td>0.42 ± 0.17</td>
<td>1.87 ± 0.85</td>
<td>0</td>
<td>0.0057</td>
</tr>
</tbody>
</table>

\( p \) values:

| (1)v.(2)v.(3) | 0.000021 | 0.000096 | 0.000036 | —       |
| (1)v.(2)      | 0.016    | 0.90     | >0.10    | —       |

| (4) +/Ph      | 57.1 ± 48  | 55 ± 10  | 24.1 ± 5.9  | 0.021   |
| (5) \( W^* +/ + Ph \) | 169 ± 25    | 77 ± 20  | 210 ± 25    | 0.013   |

\( p \) values:

| (1)v.(2)v.(3)v.(4)v.(5) | 0.00000026 | 0.0000034 | 0.000046 | —       |
| (1)v.(4)       | 0.043 | 0.73     | >0.10    | —       |
| (1)v.(5)       | 0.0095 | 0.76     | 0.0079   | —       |

### Table 2. Number of Mast Cells per Square Micrometre in Three Tissues
of Three Genotypes

(Mean ± s.e.)

<table>
<thead>
<tr>
<th>genotype</th>
<th>(a) gut</th>
<th>(b) skin</th>
<th>(c) spleen</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) +/+</td>
<td>69.5 ± 3.0</td>
<td>65.4 ± 8.1</td>
<td>4.275 ± 0.80</td>
<td>0.024</td>
</tr>
<tr>
<td>(2) +/( m^i )</td>
<td>63.7 ± 4.9</td>
<td>69 ± 20</td>
<td>0</td>
<td>0.0063</td>
</tr>
<tr>
<td>(3) ( m^i/m^i )</td>
<td>8.66 ± 0.85</td>
<td>113 ± 27</td>
<td>0</td>
<td>0.000019</td>
</tr>
</tbody>
</table>

\( p \) values:

| (1)v.(2)v.(3) | 0.0016 | 0.63 | 0.00067 |

the values in gut were significantly reduced. On this genetic background the normal spleen gave notably low values.

Radiation chimeras, \( W^e/W^e \) mice lethally irradiated (900 rad of X-rays) and treated with normal bone marrow (C3H/HeH) were restored to normal values for mast cells in gut and skin (cf. Kitamura et al. 1979b), confirming the origin of mast cells as haematopoietic stem cells.

Radiation chimeras based on \( m^i/m^i \) mice X-rayed with 600 rad of X-rays and
treated with marrow from compound $W$ mutants retained mast cells in gut and skin. This accords with a long life for host's mast cells with replacement dependent on local factors (Kitamura et al. 1979a). Spleen was not examined as no change was expected.

At no time during the two years of the study was peptic ulceration seen in the stomachs of doubly $W$ mutant mice either at necropsy or, after the report of Shimada et al. (1980), in histological sections. The faeces of these mice were consistently negative on test with Haemoccult, though normal mice fed on wet mash containing mouse blood react positively. However, some double mutants, notably $W^e/W^e$, developed necrotizing dermatitis associated with *Staphylococcus pyogenes* in affected skin and local lymph nodes (cf. Murphy 1977).

**Discussion**

Mast cells were grossly deficient in all three tissues in all combinations of two $W$ mutants, but only marginally affected in heterozygotes. Altogether the results indicate that the $W$ locus is one controlling mast cell population of the various tissues and that the mutants act recessively in this respect. For the spotting effect on coat colour, on most backgrounds $W$ mutants are commonly described as dominant, although probably truly recessive os semi-dominant (Grüneberg 1952; Silvers 1979).

$W^{sh}/W^{sh}$ homozygotes, unique in being non-anaemic, fully viable and fertile and having normal radiosensitivity (Loutit et al. 1982), were as deficient in mast cells as other double mutants. The association of mast cell and pigment defects, therefore, is closer than that for other pleiotropic effects attributable to the complex $W$ locus.

The $Ph$ gene, closely linked to $W$, had little observable effect on tissue mast cells, though a case may be made for its being to some extent complementary with $W$ in controlling erythropoiesis in radiosensitivity (Loutit 1982).

By contrast, the $mi$ gene, unrelated to $W$ and $Ph$, but with independent effect on coat colour and spotting, did have effects on the distribution of mast cells. The effect of $mi$ on mast cells in spleen appeared to be dominant. The 'significant' effect on mast cells in gut, if real, is recessive. The observations from this mutant suggest that spleen is an exceptional tissue and accord with the view that mast cells, or those cells stainable with toluidine blue, may be a heterogeneous population (Dexter et al. 1981). As mast cell populations differ in quantity and behaviour in various tissues, no tissue can be taken as representative.

That peptic ulceration was not seen in Harwell stocks with two $W$ mutants suggests that mutation at the $W$ locus by itself has no direct causative genetic effect. There may be complementation with other genes in the Jackson stock, absent in Harwell stock. There may be a general effect on various epithelia in so far as ulceration of skin was not infrequent on our mutant stock with or without relation to mast cells. Environmental factors could be of major importance.
J. F. L. thanks the Medical Research Council for a grant to pursue this work. We are greatly indebted to Mr D. Papworth for the statistical analyses, to Mr R. Evans of the U.K.A.E.A. for bacteriological assay and to Professor Y. Kitamura, a most helpful correspondent.

REFERENCES


