A Locus affecting Circulating Interferon Levels induced by Mouse Mammary Tumour Virus

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SUMMARY

Mendelian analysis, combined with the use of recombinant inbred strains, has revealed the existence in the mouse of a locus with a quantitative effect on circulating interferon levels induced by mouse mammary tumour virus. This locus segregates independently from the locus that affects NDV-induced interferon levels (Ifl) and has been tentatively designated as If-2.

There are presently no selective inhibitors of interferon synthesis for animal studies, and this makes an evaluation of the role of interferon very difficult. Another approach would be to study mutants that have lost the ability to make interferon, but such mutants have never been found, and it may well be that they are not viable. The best approach available then is to study genes that control different levels of interferon production. For this reason we are looking for genes in the mouse that influence levels of virus-induced circulating interferon, and the present communication describes the existence of such a gene for mouse mammary tumour virus (m-MTV). Using purified preparations of m-MTV, obtained either from C3H mouse milk or from C3H mammary tumour tissue, De Maeyer et al. (1972) have recently been able to induce significant levels of circulating interferon after intravenous inoculation of the virus into mice. In the course of these experiments it was observed that mice of different m-MTV resistant C57BL lines produced on the average about three times as much interferon as did mice of two different m-MTV susceptible BALB/c lines. In view of the fact that genes affecting interferon levels have been discovered for other virus inducers (De Maeyer & De Maeyer-Guignard, 1969, 1970), the difference observed with m-MTV induction was further explored, and a genetic analysis was attempted, the results of which are summarized in this communication.

For the present study, the source of virus used was C3H/He skim milk containing B-particles (its titre as measured by nodule-inducing capacity in C3H mice was $10^4$), while B-particle negative C57BL milk was used as a control (both obtained from Dr Walter E. Heston, NCI, Bethesda, Md). For interferon induction, 0.05 ml of milk containing virus or of control milk were inoculated intravenously into the retro-orbital sinus of the mouse. As in the case of purified virus, maximal serum interferon levels appeared 6 to 7 h later; serum of mice inoculated with control milk showed either no antiviral activity, or occasionally a slight activity at a 1/20 dilution. All interferon assays were carried out in secondary cultures of Swiss mouse embryo fibroblasts, using a plaque reduction assay with vesicular stomatitis virus as challenge (De Maeyer et al. 1972). Interferon units are expressed as the log$_{10}$ value of the 50% plaque reduction dose; this is a necessary condition to make statistical analysis possible, since circulating interferon titres have been found to be normally distributed on the logarithmic scale (De Maeyer & De Maeyer-Guignard, 1969). Table 1 summarizes the results obtained in BALB/c, C57BL/6, F1, F2 and backcross generations. There was a difference of 0.5 log$_{10}$ units between the mean BALB/c and C57BL/6 value, with overlapping of individual titres between both strains. The value halfway between high
## Table 1. m-MTV-induced serum interferon levels in parents and hybrids

<table>
<thead>
<tr>
<th>Strain or cross</th>
<th>BALB/cBy</th>
<th>C57BL/6By</th>
<th>BALB/c × C57BL/6</th>
<th>F₂</th>
<th>Backcross C57BL/6 × F₁</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of mice tested</td>
<td>23</td>
<td>18</td>
<td>6</td>
<td>24</td>
<td>16</td>
</tr>
<tr>
<td>Mean interferon titre</td>
<td>2.13*</td>
<td>2.62</td>
<td>2.09</td>
<td>1.95</td>
<td>2.54</td>
</tr>
<tr>
<td>High or low producer</td>
<td>Low†</td>
<td>High</td>
<td>Low</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>No. in group</td>
<td>23</td>
<td>18</td>
<td>6</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>Range</td>
<td>1.65–2.62</td>
<td>2.00–3.12</td>
<td>1.47–2.77</td>
<td>1.29–2.36</td>
<td>2.50–2.65</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.31</td>
<td>0.30</td>
<td>0.51</td>
<td>0.36</td>
<td>0.07</td>
</tr>
</tbody>
</table>

* All titres are expressed in log₁₀ units.
† Cut-off value is 2.38 (see text).

## Table 2. m-MTV-induced serum interferon levels in recombinant-inbred lines and in one congenic strain

<table>
<thead>
<tr>
<th>RI line</th>
<th>CXBD</th>
<th>CXBE</th>
<th>CXBG</th>
<th>CXBH</th>
<th>CXBI</th>
<th>CXBJ</th>
<th>CXBK</th>
<th>B6.C-H-28c</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of mice tested</td>
<td>11</td>
<td>19</td>
<td>6</td>
<td>14</td>
<td>9</td>
<td>9</td>
<td>16</td>
<td>5</td>
</tr>
<tr>
<td>Mean interferon titre</td>
<td>2.11</td>
<td>2.50</td>
<td>2.69</td>
<td>2.21</td>
<td>2.53</td>
<td>2.40</td>
<td>2.12</td>
<td>2.63</td>
</tr>
<tr>
<td>Range</td>
<td>1.65–2.67</td>
<td>1.80–2.77</td>
<td>2.28–3.04</td>
<td>1.69–2.62</td>
<td>2.02–2.77</td>
<td>1.84–2.67</td>
<td>1.60–2.57</td>
<td>2.35–2.81</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.31</td>
<td>0.21</td>
<td>0.31</td>
<td>0.24</td>
<td>0.21</td>
<td>0.25</td>
<td>0.30</td>
<td>0.20</td>
</tr>
<tr>
<td>SDP* of m-MTV interferon production (If-2)</td>
<td>l*</td>
<td>h†</td>
<td>h</td>
<td>l</td>
<td>h</td>
<td>h</td>
<td>l</td>
<td>h</td>
</tr>
<tr>
<td>SDP of NDV interferon production (If-1)</td>
<td>l</td>
<td>l</td>
<td>h</td>
<td>h</td>
<td>l</td>
<td>l</td>
<td>h</td>
<td>1</td>
</tr>
</tbody>
</table>

* l = low or BALB/c type response.
† h = high or C57BL/6 type response.
‡ SDP = strain distribution pattern.

and low producers was 2.38, and this value was arbitrarily taken as cut-off value between high and low producers for the analysis of results obtained in F₂ and backcross generations. Statistical analysis using the Student–Newman–Keuls multiple range test (Steel & Torrie, 1960) at the 5% level showed that BALB/c, F₁ and the low producer F₂ and backcross values were not significantly different; they did, however, differ significantly from C57BL/6 and high producer F₂ and backcross values. The latter three groups on the other hand did not differ significantly among one another. The fact that F₁ and BALB/c values were not significantly different indicated that low inducibility is dominant. The distribution of titres in these crosses could be explained by the effect of only one locus exerting a quantitative effect on m-MTV-induced circulating interferon levels; the fact that the average difference between high and low phenotypic groups was only 0.5 log₁₀ units and that, therefore, an arbitrary cut-off value had to be used for further analysis, obviously made this conclusion not as rigid as one would have liked. Therefore, additional evidence in favour of the single-locus hypothesis was then sought and obtained through the use of recombinant-inbred...
(RI) lines, originally derived from a BALB/c × C57BL/6 cross (Bailey, 1971). When m-MTV-induced circulating interferon levels of the seven available lines were examined and analysed at the 5% level using the Student–Newman–Keuls multiple range test, it was found that three were low producers, not significantly different from BALB/c; and four were high producers, not significantly different from C57BL/6 (Table 2). This rather clearcut response of either BALB/c or C57BL/6 type provided good additional evidence in support of the single-locus interpretation. Furthermore, the strain distribution pattern (SDP) of high and low production was different from the SDP observed for the If-1 locus, which governs the amount of interferon induced by Newcastle disease virus (De Maeyer, De Maeyer-Guignard & Bailey, 1973a). This was evidence that a different locus was responsible for the m-MTV response. Such an interpretation was further borne out by the fact that B6.C-H-28c congenic mice carrying the BALB/c allele at If-1 (De Maeyer, De Maeyer-Guignard & Bailey, 1973b), are high producers for m-MTV. We propose to designate the new locus If-2, with two alleles, If-2a for low or BALB/c type production, and If-2b for high or C57BL/6 type production. Susceptibility of mice to m-MTV-induced tumour formation is under the influence of multiple genes (as reviewed by Heston, 1972); mice of the RI lines used in this study are presently being investigated in a different laboratory for genes affecting susceptibility to m-MTV-induced tumour formation, and the outcome of that study may provide information on what role the If-2 locus might play in m-MTV oncogenesis.

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REFERENCES


