Independent Segregation of the H-2 Locus and the Locus for Responsiveness to Histamine-Sensitizing Factor

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ABSTRACT The inheritance of responsiveness to histamine-sensitizing factor of pertussis bacilli was examined in (SWR/J x CBA/J)F₁ x CBA/J backcross mice. We found that about half of the backcross mice inherited this responsiveness in both sexes. There was no linkage between the inheritance of responsiveness to histamine-sensitizing factor and the H-2b locus from the sensitive SW/J progenitor.

Responsiveness to pertussis histamine-sensitizing factor (HSF) was studied in some inbred strains of mice by Munoz and Bergman (1). Treatment with pertussis vaccine or with certain pertussis extracts renders some strains of mice susceptible to relatively low doses of histamine (treated mice die, whereas untreated controls do not). The inheritance of this responsiveness was later investigated in some of the inbred strains of mice, in their F₁ and F₂ hybrids, and in their backcrosses by Wardlaw (2). He concluded that such responsiveness is transmitted in mice by a single dominant autosomal gene.

In view of recent work showing the importance of the H-2 locus and its linkage to the immune response gene (Ir gene) (3–6) as well as to that of the genes for expression of certain tumor antigens (7, 8), the linkage relationship of the responsiveness to HSF and the H-2 locus was investigated in two inbred strains of mice. One, SWR/J strain, is sensitive to HSF; the other, CBA/J strain, is insensitive to HSF.

MATERIALS AND METHODS

Mice. SWR/J (H-2b), CBA/J (H-2a), (SWR/J x CBA/J)F₁, and (SWR/J x CBA/J)F₁ x CBA/J backcrosses of both sexes, 8–10 weeks old, were obtained from the Jackson Laboratory, Bar Harbor, Me. The mice were maintained in cages with five mice per cage and were fed Purina Chow and water freely.

HSF. HSF was prepared as described (9) and was the gift of Dr. John J. Munoz, Nat. Inst. Allergy and Infect. Dis., NIH, Rocky Mountain Lab., Hamilton, Mont. 59840. One batch was used for all experiments. HSF was dissolved in phosphate-buffered saline (9 volumes of 0.15 M NaCl + 1 volume of 0.15 M phosphate buffer [pH 7.2]). A solution of 280 µg of HSF per ml was prepared and used immediately.

Histamine. Because histamine is more stable at acid pH, it was dissolved in 0.15 M NaCl with maintenance of pH at 5.5 with 0.1 N HCl during preparation of the solution. A solution of 3.6 mg/ml was prepared and used immediately.

Injections. All mice were injected intraperitoneally with 0.5 ml of HSF solution, challenged intraperitoneally 5 days later with 0.5 ml of the histamine solution, and observed for 24 hr. Susceptible mice receiving HSF and challenged with histamine died in less than 1 hr.

Typing for H-2 Specificity. H-2 type was determined on erythrocytes by the method of Gorer and Mikulski (10), modified as described below. All mice were bled from the retroorbital sinus on the day of typing by the method of Pettit (11, 12); 0.2 ml of blood was mixed with 1 ml of heparinized 0.15 M NaCl (200 units of heparin per 10 ml of 0.15 M NaCl, Liquaemin Sodium, Organon, West Orange, N.J., 1000 USP units/ml). Erythrocytes were centrifuged for 10 min at 125 x g, washed twice with 5 ml of 0.15 M NaCl, and suspended in 0.5 ml of fetal-calf serum (previously heat-inactivated for 30 min at 56°). The typing serum (AS-656) was the gift of Dr. George D. Snell and Dr. Marrianna Cherry from the Jackson Laboratory, Bar Harbor, Me. It was prepared in (B10.A x LP.RIID)F₁ mice against B10.AKM cells taken from thymus, spleen, and submaxillary salivary glands (13). The specificity of this serum is directed against H-2b. The serum was diluted with 1.8% dextran–1.08% dextrose–saline, freshly prepared by mixing 1.8 ml of dextran–dextrose stock solution and 8.2 ml of 0.15 M NaCl. The stock solution was prepared as follows: 6 g of dextrose was dissolved in 100 ml of distilled water, and 10 g of dextran (Glaxo Laboratories, Ltd., Greenford, England, average molecular weight 110,000, batch M69/321) was added. The solution was distributed in 10-ml rubber-stoppered bottles, autoclaved for 15 min at 15 lbf pressure, and kept at 4°.

The typing serum was used at dilutions of 1/20, 1/80, and 1/320. One drop of the antiserum dilutions, or of the diluting solution for the control, was mixed with 1 drop of the erythrocyte suspension in plastic hemagglutination trays (Microtiter Plates, Cooke Engineering Co., Alexandria, Va.). The trays were covered with Scotch tape to prevent evaporation and were kept for 90 min in an incubator at 37°. Agglutination was read on glass slides 3½ x 4 inches (9.0 x 10.2 cm) by tilting the mixture and simultaneously comparing the controls with the three serum dilutions. The results are presented in Table 1.

19 out of 20 SWR/J mice died when the were injected with 140 µg of HSF and challenged 5 days later with 1.8 mg of histamine, but none of the CBA/J mice died. 22 out of 57 (SWR/J x CBA/J)F₁ x CBA/J backcross mice died. This represents 39% instead of the expected 50% fatality. There is no great difference in the death rate between those mice that inherited the H-2b allele from the sensitive strain and those that did not: 40% compared to 38% for the females and 43% compared to 36% for the males. In this respect, males and females behaved similarly. The χ² test for segregation for HSF responsiveness was 1.00 and P was >0.1.
The aim of the present study was to investigate the possibility of transmission by two genes, but only to investigate the linkage of the transmission of HSF responsiveness to the H-2 locus (to the q allele in this case).

In any case, in the backcross progeny there was about the same percentage of deaths in females (37.5%) and males (40%). Also, there was about the same percentage of deaths in those mice possessing the H-2* allele (40% in females and 43% in males) as in those not having the H-2* allele (36% in females and 39% in males).

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**DISCUSSION**

Using parental strains SWR/J and CBA/J, Wardlaw did not find differences between males and females in the F1 hybrids in development of sensivity to histamine after injection of HSF, whether or not the female parent was of the SWR/J strain (sensitive strain). All F1 mice were equally sensitive. Among the backcross progeny there was 50% mortality, which equals the expected 50% mortality if the inheritance of HSF responsiveness is unifactorial and if it is transmitted by one autosomal dominant gene. There was a slightly lower mortality in the backcross mice of the sex of the insensitive (CBA/J) parent; however, there was no significantly different ratio from 50% mortality in any case (2).

In the present experiments, the dose of HSF was uniformly 140 μg per mouse and, as Wardlaw has shown, this dose always sensitizes mice from all strains that are responsive (2). The dose of histamine (1.8 mg) was also that used by Wardlaw in the study of backcrosses between F1 and CBA/J mice.

If the HSF responsiveness were linked to the H-2 locus, it would have been expected that all those mice that died (or nearly all, to take into consideration the crossingover possibilities) should have had the H-2* allele from the SWR/J strain, but this was not the case. The χ² test for linkage of H-2* with HSF responsiveness was 0.001, and χ² was > 0.9.

The results presented here are similar to those of Wardlaw’s in SWR/J and CBA/J strains of mice. SWR/J and F1 mice died after injection of HSF when challenged with histamine. Of the backcross progeny, nearly half were sensitive to histamine when sensitized by HSF, and the remainder were not. The number of animals used was relatively small, and this could be the reason for a lower fatality (38.5% instead of the expected 50%), assuming that the HSF responsiveness is transmitted by a single, autosomal gene as Wardlaw has proposed. The small number of animals might also explain why χ² was 1.00 and P > 0.1. On the other hand, another explanation might be that a second, and perhaps nonlinked, gene is operating. The aim of the present study was not to investigate the possibility of transmission by two genes, but only to investigate the linkage of the transmission of HSF responsiveness to the H-2 locus (to the q allele in this case).

* Number of deaths per number tested.
† Expected % of deaths if the responsiveness to HSF is inherited by a single autosomal dominant gene. Segregation for HSF responsiveness χ² = 1.00; P > 0.1.
‡ Expected % of deaths if the responsiveness to HSF is linked to the H-2* allele. Segregation for linkage of H-2* and HSF responsiveness χ² = 0.001; P > 0.9.

**TABLE 1. Mortality ratio**

<table>
<thead>
<tr>
<th>H-2</th>
<th>Female</th>
<th>Male</th>
<th>% Dead</th>
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<tbody>
<tr>
<td></td>
<td>q</td>
<td>non q</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9/10*</td>
<td>—</td>
<td>10/10</td>
</tr>
<tr>
<td>SWR/J</td>
<td></td>
<td></td>
<td>19/20</td>
</tr>
<tr>
<td></td>
<td>0/10</td>
<td></td>
<td>0/10</td>
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<tr>
<td></td>
<td>10/10</td>
<td></td>
<td>20/20</td>
</tr>
<tr>
<td></td>
<td>4/10</td>
<td></td>
<td>5/22</td>
</tr>
<tr>
<td>(SWR/J x CBA/J)F₁</td>
<td>12/32</td>
<td></td>
<td>50†</td>
</tr>
<tr>
<td>(SWR/J x CBA/J)F₁ x CBA/J</td>
<td>7/18</td>
<td></td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>3/7</td>
<td></td>
<td>10/25</td>
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<td></td>
<td>—</td>
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<tr>
<td></td>
<td>22/57</td>
<td></td>
<td>50†</td>
</tr>
<tr>
<td></td>
<td></td>
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