Influence of Dietary Phenylalanine Deficiency on the Mammary Tumor Virus Activity in C3H Mice

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SUMMARY

This paper reports the blood-borne mammary tumor virus activity of C3H/Crgl female virgin mice previously fed phenylalanine-deficient diets. Whole blood was removed from three groups of female virgin C3H/Crgl mice previously fed semipurified diets containing, respectively, 0.075, 0.120, and 0.300% phenylalanine for 8 months. The blood was twice diluted and 0.3 ml was injected into each C3HfBALB/cCrgl test mouse which was then subjected to the standard noduligenesis test for viral activity. The mammary glands of each test mouse were stimulated for 12 to 13 weeks by two pituitary isografts implanted under the kidney capsule. The percentage of phenylalanine in diet of donor mice/% of donor mice bearing spontaneous hyperplastic alveolar nodules (HAN)/% of test mice bearing induced HAN are: 0.075/0/40, 0.120/16/37, and 0.300/100/39. Negative control test mice that did not receive blood but were subjected to the noduligenesis test had 6.7% incidence of HAN. The formation of spontaneous HAN in the original donor mice was completely inhibited when phenylalanine in the diet was lowered to 0.075%. However, the data reported herein show that such inhibition was not due to an absence of blood-borne mammary tumor virus. Although the relative amounts of mammary tumor virus in the donor mice was not tested, the uniformity of response of the test mice suggests that large differences would not be expected.

INTRODUCTION

We have previously reported (12–15) that phenylalanine-deficient diets can inhibit completely the formation of preneoplastic HAN2 and, partially, the transformation from nodules to tumor in the mammary glands of virgin female C3H/Crgl mice. In addition, we have demonstrated (16) that phenylalanine-deficient diets can inhibit completely the development of spontaneous preneoplastic nodules and mammary gland tumor in these mice. Both noduligenesis and tumorigenesis are dependent upon the interactions between viral, hormonal, chemical, immunological, and genetic factors (1–4, 6–11, 18–19). In an attempt to identify the mechanism of inhibition by phenylalanine deficiency, the relationship between dietary phenylalanine deficiency and MTV activity was studied.

MATERIALS AND METHODS

Diets and Animals. The experimental diets of the blood-donor mice included a semisynthetic powdered diet3 supplemented with varying levels of phenylalanine, namely, 0.075 to 0.300% (14, 16), and a mouse breeder stock food (stock diet) (14, 16).

The inbred mice were raised and housed in temperature-, light-cycle-, ventilation-controlled rooms of the animal colony of the Cancer Research Genetics Laboratory (Crgl). Weanling mice were separated into groups of 4 mice and were maintained in plastic cages. Pine shavings were used for bedding and water was given ad libitum. Stock diet was fed to all test mice.

The blood-borne viral activity (21) of mice fed 3 levels of phenylalanine and of appropriate control mice was tested. C3Hf test mice were given injections of whole blood and subjected to the standard noduligenesis test for viral activity (20).

The blood donors were virgin female C3H/Crgl mice selected from a previous study of the effect of phenylalanine-deficient diets on spontaneous nodule and mammary tumor development (16). Five groups of blood donors were selected (Table 1). One group was fed the stock diet. Three groups were fed semisynthetic powdered diets (Lofenalac) with 0.075, 0.120, and 0.300% phenylalanine, respectively. The 5th group contained pair-weight control mice that were fed restricted amounts of the 0.300% phenylalanine diet so that their body weights were equal to those of mice fed the 0.120% phenylalanine diet (16). All of these mice were 8 months old when blood samples were taken and had been fed the designated diets since 5 weeks of age. The number of mice bearing nodules in each group from which blood donors were selected is shown in Table 1.

Blood samples were taken from 7 mice in each of the 5 dietary groups (Table 1). The blood was collected in

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2 The abbreviations used are: HAN, hyperplastic alveolar nodules; MTV, mammary tumor virus.

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Table 1

<table>
<thead>
<tr>
<th>Blood donor mice (C3H/Crgl)</th>
<th>Test mice (C3HfBALB/cCrgl)</th>
<th>% HAN-bearing mice</th>
<th>No. of donors</th>
<th>No. of blood samples</th>
<th>No. of mice</th>
<th>Mice with HAN</th>
<th>Av. no. of HAN/HAN-bearing mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Stock</td>
<td></td>
<td>100.0</td>
<td>7</td>
<td>Pooled</td>
<td>35</td>
<td>15</td>
<td>43</td>
</tr>
<tr>
<td>II 0.300% phenylalanine</td>
<td></td>
<td>100.0</td>
<td>7</td>
<td>Pooled</td>
<td>36</td>
<td>14</td>
<td>39</td>
</tr>
<tr>
<td>III 0.120% phenylalanine</td>
<td></td>
<td>16.7</td>
<td>7</td>
<td>7c</td>
<td>35</td>
<td>13</td>
<td>37</td>
</tr>
<tr>
<td>IV 0.075% phenylalanine</td>
<td></td>
<td>0.0</td>
<td>7</td>
<td>7c</td>
<td>35</td>
<td>14</td>
<td>40</td>
</tr>
<tr>
<td>V Pair-weight control</td>
<td></td>
<td>46.0</td>
<td>7</td>
<td>7c</td>
<td>35</td>
<td>15</td>
<td>43</td>
</tr>
<tr>
<td>VI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15d</td>
<td>1</td>
<td>6.7</td>
</tr>
</tbody>
</table>

a Data from Ref. 16.
b Diet was supplemented Lofenalac powder to give indicated levels (%) of phenylalanine.
c Each blood sample was tested separately in groups of 4 to 6 host mice.
d Negative control group. No blood injected but mouse subjected to noduligenesis test.

RESULTS

The data in Table 1 show that blood samples taken from each of the dietary groups contained mammary tumor virus activity. Among the 176 test mice in Groups I, II, III, IV, and V, 71 (40.3%) had nodules, whereas among the 15 negative control test mice in Group VI, only 1 (6.7%) had nodules. Furthermore, each of the 21 donor mice in Groups III, IV, and V carried virus activity since nodule-bearing mice appeared in each of the 21 subgroups of test mice.

There is no relationship between the incidence of nodule-bearing mice in the 5 groups of test mice and in the 5 groups of mice which provided the blood samples. Among the test mice the mean incidence was 40.3% and the range was 37 to 43% (Table 1), whereas among the 5 groups of donor mice the incidence varied from 100% (Groups I and II) to 0% (Group IV). The incidences of mammary tumors in Groups I, II, III, and V were 73, 87, 7.7, and 11.1%, respectively, whereas no tumors occurred in Group IV after 10 months on the diet (16).

DISCUSSION

We have reported that phenylalanine-deficient diets inhibit the formation of preneoplastic nodules in C3H/Crgl mice bearing pituitary isografts (12, 14) and reduce the incidence of mammary tumors formed from nodule outgrowths (13, 15). The incidence of nodule-bearing mice and of tumor-bearing mice is greatly reduced by these diets in virgin female C3H mice (16). We have shown that the inhibition of noduligenesis and tumorigenesis is not due to permanent impairment of the mammary gland tissue (17). The data reported herein show that the inhibition was not due to an absence of blood-borne MTV. The relative amount of blood-borne viral activity in the 5 dietary groups was not tested. The uniformity of response of the test mice to a single injection of twice diluted whole blood, however, suggests that large differences in the amount of blood-borne viral activity between the 5 dietary groups would not be expected. We have suggested that diets deficient...
in phenylalanine may alter the hormonal balance of female C3H mice and inhibit the development of mammary nodules or tumor (12–17). Since the hormonal situation is one of the 4 primary factors concerned in the etiology of mouse mammary tumor system, our attempt to establish any relationship between the inhibition and blood MTV activity, the viral factor, should be regarded as a basic approach towards understanding the possible manner by which the inhibition was brought about.

ACKNOWLEDGMENTS

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REFERENCES

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