Hereditary Immunodeficiency and Leukemogenesis in HRS/J Mice


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SUMMARY

Adult and fetal tissues of hairless (hr/hr) and haired (hr/+, +/+) HRS/J mice contain the same or a similar amount of endogenous murine C-type RNA virus. However, the onset of leukemia is earlier and its incidence is somewhat greater in hr/hr than in haired mice. This finding raises the question of whether the hr mutation exerts a pleiotropic effect leading to immunodeficiency, thus rendering hairless mice more susceptible to the development of leukemia than normal mice. Several different experimental approaches yielded the following results. (a) A severe thymic cortical atrophy was observed in hr/hr mice beginning at about 6 months of age, with a concurrent increase in splenic lymphoid elements. (b) Whole-body DNA turnover as well as turnover of DNA, RNA, and protein in thymus, spleen, lymph nodes, and bone marrow were similar in all three genotypes, independent of age and sex. (c) In two in vivo test systems, phytohemagglutinin stimulation and plaque assays for sheep red cell hemolysis, the hr/hr mutant responded somewhat better than the +/+ and hr/+ animals. (d) In contrast, in intact mice, the ability to produce antibody to tetanus toxoid in the form of aluminum-adsorbed, fluid, and complexed toxoid (in slight antigen excess) was greatly reduced in hr/hr mutants. These findings indicate a relative functional defect in the immune system of these mutants. If a deficient "collaboration" among different lymphoid cell types or a deficiency in the proliferation of immunocompetent cells occurs in mutants, it may result in an ineffective immunosurveillance against leukemogenesis.

INTRODUCTION

The HRS/J strain consists of 3 genotypes (hr/hr, hr/+, and +/+ ) with respect to alleles at the hairless locus; the 1st 2 genotypes are maintained by forced heterozygosis and the 3rd was obtained by mating tested heterozygotes and by selection. Strain HRS/J originated from a cross between a random-bred strain carrying the mutation hr and the BALB/cGn strain (Ref. 28; E. L. Green, personal communication). The 3 types of HRS/J mice, +/+, hr/+, and hr/hr, are congenic, i.e., they differ only with respect to the mutant locus or a region very closely linked to it. The hairless mouse (HRS/J hr/hr) is used extensively in studies of oncogenesis (3, 27). If MuLV plays an etiological role in leukemia of HRS/J mice, the mutant gent hr or a closely linked gene might enhance the occurrence of leukemia. Conversely, the wild-type allele might confer resistance to and retard leukemogenesis despite the fact that the alleles at the hr locus do not modify the expression of complete MuLV virus. This study was undertaken to determine whether the difference observed in the incidence of leukemia among the 3 genotypes was related to a general immunodeficiency in the hr/hr mutant.

MATERIALS AND METHODS

Histological Examination of Lymphoid Organs. Thymuses and spleens from 3- and 6-month-old female HRS/J mice (The Jackson Laboratory, Bar Harbor, Maine) of the

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cells). The external standard channel ratio method was counted in a Unilux II liquid scintillation counter (Nuclear-Chicago). The external standard channel ratio method have been published in detail (18). Briefly, 50 /¿I of blood from the tail were incubated for 36 hr in 0.5 ml of toluene lOminifluor (New England Nuclear) cocktail, and the resulting dpm were corrected for the number of cells inoculated (dpm/106 cells). The activity retained was expressed as percentage of the total amount of 125I injected. From earlier studies it is known (33) that more than 80% of the 125I radioactivity present in the animal after 24 hr is stably bound to DNA, and the loss of 125I is proportional to cell death. In a 2nd experiment, leukemic mice were analyzed in the same way, but at termination of the experiment or when an animal appeared moribund, the mice were killed, autopsied, and studied histologically. Based upon the histological findings, the animals were subdivided into lymphoid and myeloid leukemia groups.

Response of Lymph Node and Thymic Cell Suspensions to PHA. The method of Adler et al. (2) was used for short-term culture of cell suspensions from mesenteric lymph nodes and thymuses of HRS/J mice. The optimal response with this system occurred at 72 hr. Labeling was with thymidine-methyl-14C (New England Nuclear; specific activity, 54.7 Ci/mM; dose, 1 /¿Ci/culture). Each culture contained 3 ml of medium, and the number of cells introduced was determined in a Neubauer counting chamber. The cultures were chased with cold thymidine (Sigma Chemical Co., St. Louis, Mo., 4 /¿g/µg/culture) and centrifuged. The cells were then washed 3 times with 2 ml of Hanks' balanced salt solution (Grand Island Biological Co., Grand Island, N. Y.) containing 4 /¿g of cold thymidine. After the last centrifugation, 2 ml of ice-cold 0.5 N perchloric acid were added and the cells were resuspended and centrifuged at 170 × g for 10 min in a Model PR-J refrigerated centrifuge (International Equipment Company, Needham Heights, Mass.). This step was repeated 3 times, followed by 2 additional washes in absolute alcohol and one in ether:alcohol. The pellets were dissolved overnight in Soluene (Packard) and counted in a Tobbor whole-body counter (Nuclear-Chicago Corp., Boston, Mass.) immediately following the injection and then at daily intervals up to 3 weeks. The activity retained was expressed as disintegrations per culture.

Assay for Transformation of Circulating Blood Lymphocytes in a Microculture System. The parameters of the method have been published in detail (18). Briefly, 50 µl of blood from the tail were incubated for 36 hr in 0.5 ml of Roswell Park Memorial Institute Medium 1640 containing 25 µl of PHA-M (Difco Laboratories, Detroit, Mich.). The cells were then cultured for 16 hr in the presence of 1 µCi thymidine-5-3H (New England Nuclear; specific activity, 2 Ci/mM). The erythrocytes were lysed by addition of distilled water and the cultures were harvested on glass filters in a Model 3025 Millipore sampling manifold (Millipore Corp., Bedford, Mass.). The macromolecules trapped on the filter after perchloric acid and alcohol precipitation were dissolved overnight in Soluene (Packard) and counted in toluene: Omnifluor (New England Nuclear); external standard channel ratio correction was used to account for quenching, and results were expressed as disintegrations per culture (i.e., dpm/50 /¿l blood).

Antitoxin Responses to Tetanus Toxoid. Two- and 8-month-old mice of all 3 genotypes and both sexes were given a s.c. primary immunization in the left flank with 0.05 ml aluminum phosphate-adsorbed tetanus toxoid (Lederle Laboratories, Pearl River, N. Y.), and sera were obtained 14, 28, and 42 days after the primary immunization. Forty-nine days after primary immunization, an s.c. booster injection of 0.1 ml FTT (Lederle Laboratories) was given, and secondary response sera were obtained 7 days later.

Hemolysis in Gel (Plaque) Assays for Cellular Responses to SRBC. The techniques for the plaque assay have been described previously (25). The mice were sensitized by an i.p. injection of 0.1 ml of 10% SRBC suspended in 0.15 M NaCl. The assays were for sensitized spleen cells from mice killed 4 or 5 days after the SRBC injection. The HRS/J strain of mice has been determined to be antigenically H-2k, Thy-1b (OC3H), and TL- (38).

Anti-OC3H antibodies were obtained either in serum or ascites fluid of AKR/J [H-2b, Thy-1b (6AKR)] mice given i.p. injections of strain C58/J (H-2k, Thy-1b) thymus cells and Freund's complete adjuvant (Difco Laboratories). Serum was obtained from bleedings made after 2 booster injections of thymic cells. At a dilution of 1:8 (plus rabbit complement), the serum killed 66% of the A/J (Thy-1b) thymocytes. The ascites fluid was taken by serial tappings of the distended abdomens. The 1st ascites sample was collected 1 week after the mice were given the last of 4 injections of thymus cells over a 2-week period and 3 injections of Freund's complete adjuvant over 1 week. The fluid killed 70% of the A/J target thymocytes at a dilution of 1:32. The antibodies are directed against TL-1,2,3 as well as OC3H, and against several non-H-2 histocompatibility antigens.

Imuran (Burroughs Wellcome & Co., Research Triangle Park, N. C.) was administered i.m., 0.2 ml in each calf muscle, at a concentration of 2 mg/ml in 0.15 M NaCl, pH 8.5. Control mice received an equal quantity of the vehicle. The
schedules of injections are given with each experiment.

RESULTS

Histological Findings. Young mice of the 3 genotypes have normal thymic and splenic architectures (Fig. 1, a and b). The thymic cortex and medulla are readily distinguishable, and the ratio of white to red pulp of the spleen appears to be normal (Fig. 2, c and d). However, the thymus of the mutant hr/hr genotype undergoes a marked cortical atrophy beginning at about 6 months of age (Fig. 2b). In contrast, the splenic follicular apparatus is both enlarged and near confluent, leading to a considerable disparity between the white and red pulp (Fig. 2d). Thus, the hairless gene exerts a direct or indirect pleiotropic effect on both the thymus and spleen, causing a disturbance in the development of these 2 lymphoid organs.

Whole-Body DNA and Macromolecular Turnovers in Single Organs. The results are shown in Fig. 3. During the critical time period, between Days 1 and 8, no significant differences in the turnover of 125I bound to DNA was found, although the curves separated between Days 8 and 12 (Chart 1A). Assays of skin, fur, and carcass showed about 50% of the radioactivity located in and on the skin of the haired genotypes (+/+ and hr/ + ), whereas only 25% was present in and on the skin of hairless animals. Therefore, the difference was interpreted as being due to 125I surface contamination by urine and fecal material. Thus, the 3 genotypes did not differ grossly in their DNA turnover as a result of cell proliferation and differentiation.

There was no difference between the turnover curves of normal and leukemic animals. From Day 8 on, however, the curves separated. Myeloid leukemic animals steadily continued to clear the DNA-bound radioactivity, whereas the curve for mice with lymphoid leukemia leveled off as it did for healthy animals (Chart 1B).

DNA, RNA, and protein turnovers were determined in spleen, thymus, lymph nodes, and bone marrow of all 3 genotypes subdivided according to sex and 2 age groups, namely, 2 and 8 months. Three different specific radioactive precursors were used, and the organs were fractionated by the method of Schmidt-Thannhauser (36). Radioactivity was assayed daily over a period of 8 days for each specific fraction. No statistically significant differences were found, either between genotypes or between sexes. Old animals appeared to have a slower turnover, compared with young mice, but the 3 genotypes behaved similarly. The data for hr/hr animals are plotted in Chart 2. DNA turnover was essentially identical to the previously noted values for C57BL/6 Rij mice (17). RNA and protein turnover showed considerable experimental variation, and the fact that their turnover was slow suggests a high rate of reutilization of either labeled precursor or label alone (11).

Response of Cell Suspensions from Lymph Nodes and Thymus to PHA. Thymic cell suspensions responded only weakly or not at all to PHA; similar findings have been reported elsewhere (4). In contrast, the lymphocytes from mesenteric nodes responded consistently to the lectin, and hr/hr mice tended to respond better than nonmutants (Chart 3). The difference, however, was not statistically significant (p > 0.1). Whereas the +/+ genotype mice responded best in only 1 experiment, the response of 8-month-old animals was generally better than that of 6-week-old animals (Fig. 5).

PHA Response of Peripheral Circulating Lymphocytes. Circulating lymphocytes in whole-blood cultures of HRS/J mice were found to undergo blastoid transformation and mitosis upon stimulation with PHA, but in comparison with other strains of mice (18), strain HRS/J was a poor responder. The hr/hr genotype responded consistently highest (Table 1), whereas no difference was found between the heterozygote (hr/+) and homozygote (+/+). The difference between the recessive (hr/hr) homozygote and the other 2 genotypes appeared significant, at least for the older animals. Young (6 weeks) animals responded weakly, and no differences were detected among the 3 genotypes. This finding indicated that in older hr/hr animals a relatively higher proportion of cells circulate that are capable of responding to the mitogen.

Responses to Tetanus Toxoid. Compared with early and good responder strains, e.g., C57L/J or BNL-Swiss, all 3 HRS/J genotypes responded slowly and poorly in their primary tetanus antitoxin responses (Chart 4). Young animals (8 weeks) produced only minute amounts of toxin-neutraliz-
Chart 2. Example of macromolecular turnover in HRS hr/hr mice. The curves are representative of the thymus of all 3 genotypes. The low turnover rates of RNA and protein, in connection with considerable fluctuation from day to day, suggest a high rate of reutilization of the radioactive precursor. Although spleen and lymph nodes differed in their turnover rates from that of the thymus as well as from one another, no difference was detected between the 3 genotypes. UdR, uridine, IUdR, iododeoxyuridine.

ing antibody at Day 14 after immunization. The observed differences with the primary responses were not evident after a booster injection of fluid toxoid, i.e., the HRS/J mice responded with normal secondary antitoxin responses.

When the primary antibody response was elicited with FTT (Table 2), no antitoxin could be detected in the sera of hr/hr and hr/+ genotypes until Day 42. HRS/J +/+ mice responded like the heterozygote (hr/+). In the BNL-SWiss random-bred mice, antibody was detectable at 28 days. At Day 42 the hr/hr mutant had a 3-fold lower response than the hr/+ control. Following a booster injection, the difference between the mutant and control was 20-fold.

Immunization with a toxoid-antitoxin complex formed in slight antigen excess markedly enhanced the primary responses in BNL-SWiss mice (Table 2). An enhanced primary response to toxoid in complex with specific antibody has been reported previously (41). Although minimal responses were obtained in hr/+ mice at day 28, no antibody was detectable in hr/hr animals at this time; yet the mutants re-
sponded well to a booster injection of FTT.

Plaque Assays for Anti-SRBC Responses. Responses were first compared between untreated hr/hr and +/+ mice (Table 3, Experiment 2); the hairless mice were 26 weeks old and the +/+ mice were 28 weeks old. The mean plaque count was again higher for the hairless mice although statistically not significant by t test (t = 2.195; p > 0.05) due to the large standard errors of the means for this experiment.

No significant differences between Imuran-treated and untreated mice were noted for either the hr/hr or +/+ groups (Experiments 3 and 4). The hairless mice given Imuran received 0.2 ml per injection 3 times per week for a total of 14 injections, starting 31 days before SRBC sensitization and ending 1 day afterward. The +/+ mice received 0.2 ml in each calf muscle (0.4 ml per treatment) 3 times weekly for a total of 16 injections, starting 34 days before SRBC sensitization and ending 1 day later.

The most striking decrease in plaque counts followed treatment with AKR anti-Thy-1-2-antibody either from ascites fluid (Experiments 7 and 8) or serum (Experiment 9). In the 3 experiments, 10 daily injections of either antibody or normal serum were started 6 days before sensitization with SRBC and ended 3 days afterwards. In Experiments 7 and 8, the amount of ascites fluid given was 0.2 ml/injection, and the mice were killed for assay 5 days after SRBC sensitization in Experiment 7, and 4 days afterwards in Experiment 8. In Experiment 9, 0.3 ml of antiserum was given per injection, and the mice were killed 5 days after SRBC sensitization. The mice were 34 weeks of age in Experiment 7, 30 weeks old in Experiment 8, and 17 weeks old in Experiment 9. The differences observed in plaque counts in Experiment 8 as compared with Experiments 7 and 9 were due to the time intervals at which the spleens were collected after SRBC sensitization (4 days versus 5 days). Jerne et al. (24) noted a peak in plaque production at Day 4 and a sharp drop thereafter. Our findings are in accord with this report.

Chart 3. Response of cell suspensions from spleens to PHA in HRS/J mice of 2 different age groups. In both groups, hr/hr mice tended to respond best, although the difference was not statistically significant.

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Table 1
Response of circulating blood lymphocytes to PHA stimulation in HRS/J mice

Response to PHA in whole-blood microculture. In young and old mice, the hr/hr mutants performed best in terms of absolute counts and stimulation index.* At 4 to 8 weeks of age, the responses were low and the difference between the genotypes was significant only in males (p < 0.05). In older animals, the difference was significant (p < 0.05) in both sexes.

Table 1

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Treatment</th>
<th>4-8 wk old</th>
<th>36-48 wk old</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>+/+</td>
<td>PHA</td>
<td>7,595 ± 2,000</td>
<td>2,188 ± 253</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>1,677 ± 434</td>
<td>1,190 ± 192</td>
</tr>
<tr>
<td></td>
<td>SI</td>
<td>4.53</td>
<td>1.84</td>
</tr>
<tr>
<td>hr/+</td>
<td>PHA</td>
<td>9,093 ± 938</td>
<td>2,327 ± 627</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>2,172 ± 983</td>
<td>2,599 ± 1,192</td>
</tr>
<tr>
<td></td>
<td>SI</td>
<td>4.19</td>
<td>0.90</td>
</tr>
<tr>
<td>hr/hr</td>
<td>PHA</td>
<td>7,740 ± 2,067</td>
<td>12,335 ± 5,585</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>994 ± 349</td>
<td>1,993 ± 996</td>
</tr>
<tr>
<td></td>
<td>SI</td>
<td>7.79</td>
<td>6.20</td>
</tr>
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</table>

*SI = stimulation index = PHA-treated mice/controls.

Chart 4. Response of HRS/J mice against tetanus toxoid in young (A) and older (B) animals. For comparison, the responses of 2 high responder strains (C57L/J and BNL-Swiss) are plotted in A. In 8-month-old HRS/J mice, no titer was detectable at 14 days after injection of aluminum phosphate-adsorbed tetanus toxoid. At Days 28 and 42, hr/hr mice exhibited only one-half the titer observed in haired genotypes. This difference was not evident in secondary responses elicited at Day 49 with FTT.

DISCUSSION

It is not known how gene products interfere with or potentiate the processes of cell differentiation or malignant transformation. One important mechanism may relate to the reticuloendothelial system, i.e., involve immunological pathways (3, 27). The differences we observed in the 3 genotypes of HRS/J mice may stem from disturbances among immunocompetent cells. An early disturbance of normal thymus development and involution during adult life of mutant mice is evidenced by disproportion between the hypotrophic cortex and the normal medulla region of hr/hr mice (Fig. 2). The cortical region in the adult is abnormally small, compared with the medulla, but cortical areas were present. Correspondingly, the white pulp in the spleen of hr/hr mutants is unusually enlarged and shows abnormal germinal centers which often are confluent. Despite these findings, no major differences in the kinetics of macromolecular turnover were detected in these organs. First, the overall DNA turnover as measured by UdR-125I was very similar in all 3 genotypes. Second, our attempts to demonstrate differences in single organs by biochemical methods failed to reveal abnormalities in cellular kinetics. Nevertheless, we cannot exclude the possibility that different generation times of the cell cycles, as determined by the mitotic labeling index method (5), may be found later.

The rather nonspecific proliferate stimulation of lymphoid cells by PHA requires comment as to the ability of peripheral lymphoid cells to react to an antigenic stimulus (29, 34). Two experimental methods that do not yield identical results are in general use. The 1st uses cell suspensions prepared from lymphoid organs such as thymus, lymph nodes, and spleen (2, 29); the 2nd type of method mainly analyzes circulating lymphocytes in the blood (15, 18). Both methods complement each other. With the 1st method, cells of hr/hr mice seemed to respond at least as well as those of hr/+ and +/+ mice when suspensions from lymph nodes were used. The fact that suspensions from thymus did not give any appreciable response suggests that these cells have not acquired the capacity to react to PHA. The reactivity of lymphoid cells was somewhat more pronounced for circulating lymphocytes of the blood (2nd culture method)
Table 2

Antitoxin responses elicited with FTT and complexed toxoid

Response of HRS/J mice against different forms of tetanus toxoid. Whereas, with FTT, hr/+ and hr/hr mice show only very low titers of antitoxin, the hr/hr mice are not able to respond better to CPX, as do hr/+ mice. Wild-type mice (+/+ ) were found to respond as do hr/+ animals. The secondary response is weak in hr/hr mice after FTT primary injection, but there is no difference between the 2 genotypes in the secondary response when CPX is used as a primary injection.

<table>
<thead>
<tr>
<th>Group</th>
<th>Strain</th>
<th>Primary immunization</th>
<th>MLDa of neutralized toxin/ml pooled sera</th>
<th>Secondary responses 7 days postbooster (× 10³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(0.2 ml s.c.)</td>
<td>Day 14</td>
<td>Day 28</td>
</tr>
<tr>
<td>1</td>
<td>BNL-Swiss</td>
<td>FTT</td>
<td>0</td>
<td>38</td>
</tr>
<tr>
<td>2</td>
<td>HRS/J (hr/+)</td>
<td>FTT</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>HRS/J (hr/hr)</td>
<td>FTT</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>BNL-Swiss</td>
<td>CPX</td>
<td>12.5</td>
<td>1900</td>
</tr>
<tr>
<td>5</td>
<td>HRS/J (hr/+)</td>
<td>CPX</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>HRS/J (hr/hr)</td>
<td>CPX</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>BNL</td>
<td>Mouse IgGc</td>
<td>200</td>
<td>12</td>
</tr>
</tbody>
</table>

a Complex of FTT and specific mouse IgG in slight antigen excess.

Table 3

Hemolysis in gel (plaque) assays for SRBC response in spleen cells of HRS/J mice

The plaque counts are higher in hr/hr animals, compared with +/+ mice, in Experiment 2. No differences between the 2 genotypes were found in Experiments 3 and 4 (Imuran treatment). Anti-0C3H ascites fluid reduced the plaque counts drastically in hr/hr mice and to a minor extent in +/+ mice (Experiments 7 and 8). In +/+ mice, anti-0C3H serum (Experiment 9) had an even larger effect than anti-0C3H ascites fluid.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Genotype</th>
<th>Day mice killed after SRBC injection</th>
<th>Treatment</th>
<th>No. of plaques/10⁶ spleen cells</th>
<th>p*</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>hr/hr (3 F)</td>
<td>4</td>
<td>None</td>
<td>335 ± 95.7c</td>
<td>N.S.a</td>
<td>2.195</td>
</tr>
<tr>
<td>3</td>
<td>+/+ (3 F)</td>
<td>4</td>
<td>None</td>
<td>137 ± 23.5</td>
<td>N.S.</td>
<td>1.584</td>
</tr>
<tr>
<td>4</td>
<td>hr/hr (3 F)</td>
<td>4</td>
<td>Imuran</td>
<td>611 ± 155.4</td>
<td>N.S.</td>
<td>0.828</td>
</tr>
<tr>
<td>5</td>
<td>+/+ (3 F)</td>
<td>4</td>
<td>Imuran</td>
<td>357 ± 39.3</td>
<td>N.S.</td>
<td>0.828</td>
</tr>
<tr>
<td>6</td>
<td>+/+ (3 F)</td>
<td>4</td>
<td>None</td>
<td>681 ± 196.1</td>
<td>N.S.</td>
<td>0.828</td>
</tr>
<tr>
<td>7</td>
<td>hr/hr (3 F)</td>
<td>5</td>
<td>Anti-0C3H ascites fluid</td>
<td>8 ± 1.0</td>
<td>&lt;0.001</td>
<td>9.690</td>
</tr>
<tr>
<td></td>
<td>hr/hr (3 F)</td>
<td>Normal ascites fluid</td>
<td></td>
<td>102 ± 9.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>+/+ (3 M)</td>
<td>4</td>
<td>Anti-0C3H ascites fluid</td>
<td>227 ± 27.7</td>
<td>&lt;0.1</td>
<td>5.235</td>
</tr>
<tr>
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<td>+/+ (3 M)</td>
<td>Normal ascites fluid</td>
<td></td>
<td>701 ± 86.2</td>
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</tr>
<tr>
<td>9</td>
<td>+/+ (3 F)</td>
<td>5</td>
<td>Anti-0C3H serum</td>
<td>12 ± 2.7</td>
<td>&lt;0.001</td>
<td>6.050</td>
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<td>+/+ (3 F)</td>
<td>Normal serum</td>
<td></td>
<td>169 ± 25.9</td>
<td></td>
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</tbody>
</table>

* By Student's t test.

* Numbers and letters in parentheses, number and sex of mice.

* Mean ± S.E.

* N.S., not significant at the 0.05 level.

than for lymph node cells. This indicates that a relatively larger number of cells capable of responding to PHA circulate in hr/hr mice. It has been proposed that the circulating cell that responds to PHA may be thymus dependent (2, 9). Thymus-independent cells (so-called B-cells) reportedly respond to a lectin, e.g., pokeweed mitogen (39), although the
methods for obtaining pure cell populations are still equiv-
ocal in terms of their selectivity.

The HRS/J strain responded very poorly to tetanus tox-
oid as compared with the C57L/J and BNL-Swiss strains.
Nevertheless, the 3 genotypes of young HRS/J mice (Fig.
6) produced detectable antibody at 14 days after primary
immunization with adsorbed toxoid. This ability was lost in
8-month-old HRS/J mice, and the hr/hr mutant was clearly
the slowest and weakest responder. This is concurrent with
the time when morphological changes in the thymus are
prominent, the 1st leukemias appear, and changes in re-
response to PHA are noted. Conversely, secondary antitoxin
responses were not impaired in the HRS/J genotypes; these
animals responded to a booster of FTT almost as well as the
high primary responder strains (C57L/J or BNL-Swiss).
Since the 3 genotypes of the HRS/J strain are poor re-
sonders, it could be presumed that the extreme low re-
response in hr/hr animals may be unrelated to the hairless
locus. However, the difference in response between hr/hr
and hr/+ or +/+ was reproduced in several experiments
and we, therefore, must consider it significant. Thus, the hr
locus further diminishes the response to tetanus toxin on a
genetic background that has already made the animals poor
responders. When toxoid was administered in fluid form
rather than as adsorbed toxoid for primary immunization
(Table 2), the HRS/J mice did not respond with detectable
primary responses until day 42 after primary immunization.
Unfortunately, not enough homozygous wild-type animals
(+/+ ) were available for this experimental series. However,
in none of our experiments is there evidence for any differ-
ence between the +/+ and the hr/+ animals, i.e., no gene
dose effect is detectable. Ordinarily, primary responses to
FTT are slow and rather poor in humans, and even in good
primary responding strains of mice. However, when toxoid
was complexed with specific mouse IgG for the tox-
oid and injected in slight antigenic excess, the BNL-Swiss
mice produced an extremely high primary antitoxin titer
(41). In contrast, the HRS/J hr/hr mice did not respond
any better to the complexed toxoid than to the same amount
of toxoid given alone. This finding suggests that the ob-
served immunodeficiency in HRS/J mice may be due either
to an inability to recognize an antigen or to proliferate spe-
cific antibody-producing cells. If the defect is an inadequ-
ate proliferation of immunocompetent cell precursors, then
it can be assumed that no differences would be found between
the various forms of toxoid used, i.e., adsorbed, fluid, and
complexed toxoid. Indeed, good reactors such as BNL-
Swiss and C57L/J mice respond well to complexed FTT
and to adsorbed toxoid. The hr/hr mutant mice were able
to produce measurable primary antibody titers only against
adsorbed toxoid. It should be noted that adsorbed toxoid
persists as a deposit for many weeks, thus providing con-
tinuous antigenic stimulation. This finding is in agreement
with our failure to detect differences in macromolecular
turnover resulting from cellular proliferation. Nevertheless,
until kinetic data on the cell cycles of various lymphoid cell
types in the 3 genotypes are available, disturbances of the
proliferation pattern within certain cell populations cannot
be ruled out.

The SRBC plaque assay (24, 25) has been considered as a
measure of early IgM antibody production in thymus-in-
dependent or so-called B-cells. Imuran probably interferes
with the proliferation of lymphoid precursor cells (30), al-
though other observations point to direct effects on the so-
called B-cells (1). In this study, Imuran severely suppressed
the immune response of HRS/J mice against injected tumor
cells (15091A) of the H-2a type when circulating anti-H-2a
antibodies were assessed by a hemagglutination test (N.
Kaliss, unpublished results). Yet, in our SRBC plaque
assays, no statistically significant effects of Imuran treat-
ment in the various genotypes were observed (Table 3). In
contrast, we found a severe suppression of the plaque re-
sonse when thymus-dependent lymphoid cell populations
were destroyed in vivo by anti-θC3H antibodies. These find-
ings are in line with the results of all other tests described;
they suggest that the basic defects in hairless mice may re-
side in thymus-dependent cell types, and more likely are
connected with the process of primary antigen recognition
rather than with cell proliferation. Thus, this mechanism
may be of significance relative to the genetic differences ob-
served in leukemogenesis between the mutant and nonmut-
ant HRS/J mice.

In our experiments, the hr/hr mutant responded some-
what better than did the wild type in 2 experimental in vitro
conditions involving PHA stimulation and SRBC plaque
assay, whereas in an in vivo system, the tetanus-antitoxin
test, the hr/hr mouse was definitely handicapped in its pri-
mary ability to recognize tetanus toxoid as an antigen and
produce normal antitoxin responses.

No differences were observed in the expression of the
endogenous C-type RNA virus in all 3 genotypes, i.e., the,
mice are all gs-1 positive and the virus replicates to com-
parable titers (13, 30). By inference, we assume that HRS/J
mice are like AKR/J mice in this respect (42). The hr locus
or a closely linked gene shortens the time interval within
which leukemia occurs. The underlying mechanisms may
reside in unknown deficiencies of the immunological sys-
tem. From our findings, those tissues known to be affected
in one way or another in the hr/hr mutant, e.g., the skin and
the thymus, are initially at least in part derived from the
embryonal epidermal cell layer. We speculate, therefore,
that cell populations derived from these embryonal tissues
may be only partially functional perhaps due to some defect
in the inductive tissue interaction between epithelium and
mesenchyme during early embryonal development. Later
in adult life, a deficiency in collaboration among several cell
types originating in or processed by the thymus could result
in a deficient immune system and, as 1 consequence, lead
to an ineffective immunosurveillance against malignant
cells.

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Fig. 1. Cross-sections through thymus and spleen of HRS/J+/+ mice (a and c) and HRS/J hr/hr mutants (b and d) at 3 months of age. The thymic cortices are equally well developed in both genotypes, and the spleens (c+/+ d, hr/hr) show a normal morphological pattern.

Fig. 2. Cross-sections through thymus (a, +/+: b, hr/hr) and spleen (c, +/+: d, hr/hr) of 6-month-old HRS/J mice. There are obvious differences between the 2 genotypes, i.e., cortical atrophy of the thymus in hr/hr mutants (b) and large confluent splenic follicles (d), compared with the normal structures present in the organs of +/+ mice (a, c).
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