Subclasses of T lymphocytes in the mouse can be distinguished by the different Lyt components expressed on their surfaces (1, 2). The three cell sets so identified are Ly1, Ly23, and Ly123 (3), and these have different immune functions (4, 5). The Lyt surface phenotypes and corresponding functions of these cell populations are presumed to reflect discrete genetic programs (6). This immunogenetic classification of T cells provides new criteria for investigating immunological disorders, because it can be determined serologically whether there are absolute or relative changes in the representation of Lyt sets, and whether these changes are associated with alterations in immune reactivity that correspond with the established functional properties of Lyt sets. For example, Cantor and colleagues (7) propose that NZB mice are prone to autoimmune disease because they are defective in feedback inhibition, this being reflected serologically in a deficiency in the Ly123 population which is the source of suppressor T cells.

In the present study we took as our model the HRS/J mouse bearing the mutant gene \( hr \) (hairless). Homozygous \( hr/hr \) mice lose their fur in infancy, they suffer a premature decline in immune capacity (8) which may be responsible for their high incidence of leukemia in comparison with \( +/hr \) littermates (9), and their thymuses are said to show early cortical atrophy (8).

The aim of this study was to determine the influence of the \( hr \) locus on the Ly1, Ly23, and Ly123 T-cell sets.

### Materials and Methods

**Mice.** HRS/J breeding stock was obtained from the Jackson Laboratory, Bar Harbor, Maine, and the strain maintained by mating \( +/hr \) females with \( hr/hr \) males. Only mice that appeared healthy and showed no abnormal enlargement of spleen or lymph nodes were used. Some \( +/hr \) mice show various degrees of hair loss, at a later age than \( hr/hr \) segregants, suggesting that the \( hr \) mutation is not completely recessive; such \( +/hr \) mice were excluded from this study.

**Antisera.** Congenic \( \alpha\)-Thy-1.2 antiserum (A-Thy-1.1 × AKR-H-2\( ^b \) anti-A strain leukemia ASL1) was absorbed twice for 30 min on ice at a dilution of 1:5 with the Thy-1.1 AKR strain leukemia K36 (vol/vol \( = 1/1 \)). For details of the preparation and the use of Lyt antisera see Shen et al. (10). Lyt antisera were absorbed twice at a dilution of 1:2 with an equal volume of packed thymus, spleen, and lymph node cells of mouse strains negative for the respective Lyt alloantigen. As an extra check on the specificity of the absorbed Lyt antisera the control recommended by Shen et al. (10) was also employed; thus each Lyt antiserum (eg \( \alpha\)-Lyt-1.2) was tested on HRS cells after absorption with thymocytes of the same allotype (B6; all activity
TABLE I
Calculation of Lyt Sets

| Antisera | Lysed Lyt set | % lysis* |
|----------|--------------|---------|
| α-Lyt-1.2 | Lyt-1        | Lyt-123 | A       |
| α-Lyt-3.2 | Lyt-23       | Lyt-123 | B       |
| α-Lyt-1.2 + α-Lyt-3.2 | Lyt-1        | Lyt-123 | C       |

* Thus, C = total Lyt+ population; C-B = Lyt1 set; C-A = Lyt23 set; A + B - C = Lyt123 set.

should be abolished) or with thymocytes from congenic mice of the alternative allotype (B6-Lyt-1.1; no activity should be removed). Rabbit antiserum against immunoadsorbent-purified F(ab)2 of mouse IgG (11) was kindly supplied by Dr. U. Hammerling, Sloan-Kettering Institute.

Cytotoxicity Assay. Suspensions of spleen cells were treated with Tris-ammonium chloride to eliminate erythrocytes (12), and labeled with 51Cr as described (13). For the cytotoxicity assay, 2.5 × 10^6 labeled spleen cells (50 #l) were incubated with 50 #l antiserum (predetermined optimal dilution in phosphate-buffered saline), or with normal mouse serum (NMS, control) for 30 min on ice, washed once, resuspended in 100 #l freshly-thawed rabbit serum (complement source; predetermined optimal dilution), and incubated further for 30 min at 37°C. A single pool of rabbit serum selected for low natural cytotoxicity and high complement activity was used throughout. Percent lysis was calculated by the formula:

\[
\frac{\text{cpm (antiserum)} - \text{cpm (NMS)}}{\text{cpm (maximum release)} - \text{cpm (NMS)}} \times 100.
\]

Enumeration of Lyt-Cell Sets. The proportions of Lyt1, Lyt23, and Lyt123 cell sets were estimated from the percent lysis by Lyt-1 and Lyt-3 antisera used alone and in combination. Data for Lyt sets were expressed as percent of total Lyt+ cells. Because Lyt-2 and Lyt-3 have not yet been found to be expressed independently, and because α-Lyt-2.2 and α-Lyt-3.2 gave similar counts, we used the latter, which was more readily available at the time of these experiments. Table I illustrates the method.

Enumeration of B Cells. Cells bearing surface immunoglobulin (Ig) were estimated by the Protein A rosette assay.2 Cells expressing complement receptor (CR) were enumerated by rosetting with sheep erythrocytes (SRBC) coated with IgM anti-SRBC antibody and mouse complement (14). Ig+ and CR+ cells were expressed as percentage of the total nucleated cell population.

Results

Each assay was conducted with a pair of mice, one of each genotype, hr/hr and +/hr, matched for age and sex, and tested concurrently.

T- and B-Cell Sets in Spleens of HRS Mice. The relative numbers of T lymphocytes in spleens of hr/hr and +/hr mice were assessed by cytotoxicity assay with Thy-1.2 antiserum. As shown in Table II, the percentage of Thy-1+ cells was comparable in the two genotypes, increasing slightly with age. Mice of both genotypes also had similar percentages of total Lyt+ cells. The apparent excess of Lyt+ cells over Thy-1+ cells indicated by some entries in Table II may simply be due to Lyt+.Thy-1low cells which are less efficiently registered in cytotoxicity assays with Thy-1 antiserum than with combined Lyt antisera.

No difference was found in the representation of Ig+ and CR+ cells in mice of the two genotypes (Table II), both Ig+ and CR+ cells showing an increase with age, as observed in other mouse strains (15, 16).

1 Abbreviations used in this paper: CR, complement receptor; NMS, normal mouse serum; SRBC, sheep erythrocytes.

2 Scheid, M. P. Manuscript in preparation.
**T-CELL SUBPOPULATIONS IN hr/hr MICE**

**Table II**

| Lymphocyte class | Cell-surface marker | 1-1.5 Mo (Group I) | 2-2.5 Mo (Group II) | 3-3.5 Mo (Group III) | 7-10 Mo (Group IV) |
|------------------|---------------------|-------------------|-------------------|-------------------|-------------------|
|                  |                     | hr/hr +/hr        | hr/hr +/hr        | hr/hr +/hr        | hr/hr +/hr        |
|                  | % marker-positive cells in spleen ± SEM |
| T                | Thy-I               | 17 ± 1            | 21 ± 3            | 21 ± 3            | 26 ± 2            |
|                  | Lyt‡                | 18 ± 1            | 19 ± 2            | 25 ± 1            | 26 ± 1            |
| B                | Ig                  | 39 ± 1            | 46 ± 1            | 48 ± 1            | 53 ± 1            |
|                  | CR                  | 26 ± 1            | 35 ± 2            | 44 ± 1            | 44 ± 1            |

*To minimize technical variation, single pairs of hr/hr and +/hr mice, matched for age and sex, were examined in each test. The data for Thy-I and Lyt refer to tests on the same mice: There were seven pairs in group I; four pairs in group II; eight pairs in group III; and eight pairs in group IV. The Lyt cell set analysis for these mice is given in Fig. 1. Enumeration of Ig* and CR* cells was carried out on other pairs: two pairs per age group for Ig* cells and five further pairs (total seven pairs per group) for CR* cells. Only mice that appeared healthy, showed no abnormal enlargement of spleen and lymph nodes, and in the case of +/hr segregants showed no obvious hair loss, were selected for this study.

‡ Positive reactivity with α-Lyt-1 and α-Lyt-3 sera combined.

The body weights of the two groups of mice, hr/hr and +/hr, were not significantly different in any of the four age groups. The spleen weights of hr/hr mice were higher than those of +/hr mice by a factor of roughly 1.3 in all four age groups. An estimate of the number of cells recoverable in suspension from these spleens suggests that the minor degree of splenomegaly in hr/hr mice is associated with a higher number of freely dissociable cells, by a factor of roughly 1.2, for all four age groups. This suggests that the absolute numbers of lymphocytes present in the spleen of hr/hr mice may be higher than in +/hr mice, without regard to lymphocyte class (Table II).

**Proportions of Lyt Subsets in hr/hr and +/hr Spleen at Different Ages.** Fig. 1 shows the proportions of the three Lyt subsets in spleens of the two genotypes at ages ranging from 1 to 10 mo. In both hr/hr and +/hr spleens, the Lyl cells increase in number with age, and the Ly123 cells decline until ~2.5 mo of age.

Around the age of 3 mo, however, the representation of Lyt sets is dramatically different for the two genotypes, as indicated by group III in Fig. 1. In +/hr mice the increase in Ly1 cells and decrease in Ly123 cells, reported as characteristic of normal BALB/c (7) and C57BL/6 mice (reference 5, and personal unpublished data), continues. In the hr/hr group, however, the reverse occurs, Ly1 cells greatly decreasing in number, and Ly123 cells greatly increasing; (the difference between hr/hr and +/hr is statistically significant with P < 0.001 and <0.02, respectively). The low proportion of Ly1 cells in hr/hr mice reflects a relative and absolute numerical deficiency and the high proportion of Ly123 cells a relative and absolute increase.

In +/hr mice of the oldest age group (group IV; 7-10 mo), the relative proportions of Ly1 and Ly123 cells (box with interrupted line in Fig. 1) resembles the picture seen in hr/hr mice at a younger age (group III; 3-3.5 mo; box with solid line in Fig. 1).

The representation of the Ly23 set in the HRS strain, as in other strains of mice, is too small for accurate estimation by present serological methods.

The few tests we have made on T cells from pooled lymph nodes (mesenteric included) of hr/hr segregants do not indicate the same gross distortion of the Ly1:
Ly123 ratio characteristic of spleen. These data do not exclude a minor disproportion, nor have we studied the representation of Lyt subsets in particular nodes of hr/hr mice.

Discussion

The effects of hr homozygosity on the immune system can now be defined as an inversion of the normal ratio of Ly1 cells to Ly123 cells in the spleen, most evident at ≈3 mo old. The data indicate a relative and absolute deficit of splenic Ly1 cells and a relative and absolute excess of splenic Ly123 cells. Presumably these changes in the structure of the splenic T cell population account for the immunological aberrations of hr/hr mice, and provide a basis for designing and interpreting tests of immune function of cells from hr/hr mice in vitro.

Unfortunately, too few +/+ homozygous HRS segregants were available for comparison, otherwise it would have been possible to confirm our impression that the effects of hr on the representation of Lyt sets are not fully recessive, as evidently is also true of the effects of hr on the growth of hair in a few heterozygotes. Thus the low ratio of Ly1 cells to Ly123 cells in +/hr mice of the oldest age group (group IV; aged 7-10 mo), although not seen in BALB/c and C57BL/6 of the same age, could be a peculiarity of aging in the HRS strain rather than an effect of hr in heterozygotes.

The immunological aberrations of hr/hr mice might be entirely due to the disproportion in T-cell subsets, without there being any defect in functions of the cells. Because Ly123 cells are thought to be the source of Ly1 cells (5) this disproportion might connote genetic impairment of a differentiative step; and such a defect might lie in an inductive microenvironment (17), exemplified in erythropoiesis by mutations at the Sl locus which cause macrocytic anemia, rather than in the affected cells themselves, exemplified by mutations at the W locus which produce an indistinguishable anemia (18).

Summary

Mice of the HRS strain, which carry the mutant gene hr, were examined for
abnormalities in representation of the three T-cell sets Ly1, Ly23, and Ly123 in the spleen. The salient feature of hr/hr mice, which are immunologically deficient, in comparison with +/hr segregants, was a gross disproportion in numbers of cells belonging to the Ly1 and Ly123 sets, at the age of 3–3.5 mo. At this age, Ly123 cells of hr/hr spleen outnumbered Ly1 cells by 2:1, whereas in +/hr spleens Ly123 cells were outnumbered by 21:2. Cells from pooled lymph nodes of hr/hr mice did not show a correspondingly gross disporportion of Ly1 and Ly123 cells. Total counts of splenic T cells, and of B cells, were not significantly different in hr/hr and +/hr mice.

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