Although Chediak-Higashi syndrome (CHS) has been found in various mammalian species, it has not been described in rats. Since giant granules characterizing CHS are easily recognizable in mast cells of beige (CHS) mice, we screened mast-cell granules in the auricle of some mutant rats, of which coat color was diluted by mutation. Giant granules of mast cells were found in a mutant trait that occurred in the inbred colony of the DA strain maintained in Hamamatsu University School of Medicine. Giant granules were also observed in neutrophils and pigment cells of the eye. In this mutant, either spontaneous migra-

CHEDIAK-HIGASHI SYNDROME (CHS) is an autosomal recessive genetic disease that has been reported in humans, mink, cattle, mice, killer whales, and cats. CHS was initially recognized as a disorder in which melanocytes, neutrophils, and lymphocytes contained giant cytoplasmic granules. Giant granules are also remarkable in various types of cells such as mast cells, type II alveolar cells of the lung, and cultured fibroblasts. Although the molecular mechanism of CHS has not been clarified, it is now considered to be generalized cellular dysfunction characterized by increased fusion of cytoplasmic granules. Dilution of hair and skin colors in CHS results from pathologic aggregation of melanosomes. The mutation of mice comparable to human CHS is named “beige” from the diluted coat color.

Increased susceptibility observed in human CHS patients to bacterial and fungal infections can be explained, in part, by defective degranulation of abnormal neutrophil granules. The beige mutation of mice is maintained on three genetic backgrounds, i.e., SB/Le (-bg/bg), C3H (-bg/bg), and C57BL/6 (-bg/bg). Although the incidence of pneumonitis is significantly higher in SB/Le/bg/bg mice than in congenic normal (+/+) mice, the increased susceptibility to pneumonitis is not detectable in C57BL/6/bg/bg mice. However, when chemotaxis and bactericidal function of neutrophils were examined, these functions were impaired even in C57BL/6-bg/bg mice.

Human CHS patients, CHS cattle, and beige mice show prolonged bleeding with normal platelet counts, resulting from impaired platelet aggregation associated with a deficiency of the storage pools of ADP and serotonin. Furthermore, deficiency of natural killer activity of lymphocytes has been reported in both beige mice and human CHS patients.

Rats are one of the most popular laboratory animals, and appear to be more favorable animal than mice in some studies of biochemistry and chemical carcinogenesis. If CHS rats are available, they are probably useful for investigations such as biochemical analysis of CHS and the study of the significance of natural killer activity against development of cancers. Since CHS has been found in various mammalian species, we attempted to find a rat mutant comparable to CHS. We frequently use giant granules of beige mice as a marker to examine the origin of mast cells, and therefore we screened mast cell granules of some rat mutants, of which coat color is diluted. We found a rat mutant comparable with CHS and report here.

MATERIALS AND METHODS

Rats. All rat mutants are maintained in the Institute of Experimental Animals, Hamamatsu University School of Medicine, Shizuoka, Japan. The TM strain with coat color dilution was established by one of us (M.N.) as an inbred strain, and was maintained by brother-sister mating. The coat color dilution of TM strain resembles red-eyed dilute. The NIG-III strain also has diluted coat color. This strain was established by late Dr. Yoshida of the National Institute of Genetics as an inbred strain, and has been maintained by brother-sister mating. The coat color dilution of the NIG-III strain is considered to be due to the p<sup>+</sup> (ruby eyed dilute) mutant gene. The DA strain was derived from the Australian National University, and has been maintained by brother-sister mating. In spring 1985, a male rat with diluted coat color was found in this inbred colony. The male rat was crossed to a normal (agouti) female DA rat, and one of female offspring was backcrossed to the male rat with diluted coat color. Male and female offspring with diluted coat color were obtained, and then this mutant line was maintained by brother-sister mating.

Genetic examination. The specific cross to determine the inheritance of the coat color dilution is given under Results. Skin grafts were carried out with a method described previously.

Morphologic examination. Rats were anesthetized with inhalation of ether; the tip of an auricle was cut with scissors, and fixed in 10% formalin. These specimens were sent from the Hamamatsu University School of Medicine to Osaka University Medical School, Osaka, Japan.
and then embedded in paraffin; sections (5 μm thick) were stained with toluidine blue.

Blood samples were obtained from the retroorbital sinus of some DA rats with or without coat color dilution; smears were stained with Giemsa solution or Sudan Black. The DA rats were then killed by over-inhalation of ether, and eyes were removed and fixed in 10% formalin; paraffin sections were stained with hematoxylin-eosin.

Preparation of neutrophils. Neutrophils were obtained from peritoneal exudates 16 hours after intraperitoneal injection of 8 mL of sterile 10% sodium caseinate (Difco Lab, Detroit) in isotonic saline according to the method described by Gallin et al. Immediately prior to harvesting the peritoneal exudates, 10 mL of heparinized saline was injected intraperitoneally. The animals were anesthetized with inhalation of ether and sacrificed by exsanguination. The peritoneal cavity was opened, and the exudate was aspirated. Small amounts of contaminating erythrocytes were lysed by osmotic shock with NH₄Cl. The cells were washed twice and the supernatant was aspirated. Small amounts of contaminating erythrocytes were lysed by osmotic shock with NH₄Cl. The cells were washed twice in Hanks' solution. The completed sonication was -80%.

Estimation of chemotaxis. Spontaneous migration and chemotaxis under agarose plate were evaluated according to the method described by Nelson et al23 with slight modifications. Five milliliters of agarose medium were introduced to each 60-mm dish containing a suspension containing 5 × 10⁶ cells. The suspension was lysed by osmotic shock with 0.5 mL of 20 mol/L ascorbic acid to stabilize serotonin and 100 μL of 5% CO₂ in air. After incubation for 1 hour, the cells had moved from the margin of the migration control medium, ie, Hanks' solution. The completed medium was removed, and the exudate was aspirated. Small amounts of contaminating erythrocytes were lysed by osmotic shock with NH₄Cl. The cells were washed twice in Hanks' solution. The purity of granulocytes was ~80%.

Preparation of spleen cells for the assay of natural killer activity. Spleen cell suspensions were prepared by using a loose-fitting glass homogenizer, and then passed through nylon mesh. Adherent cells were removed by incubating 2 to 8 × 10⁶ cells in a column, a 30 mL disposable plastic syringe stuffed with nylon wool (1.5 g). The cells were added in a total volume (5 mL) of complete medium (PRMI 1640 medium with 10% fetal bovine serum, supplemented with 100 U penicillin, 100 μg streptomycin, 2 mmol/L guanine, and 25 mmol/L Heps) and incubated at 37°C for 60 minutes. Nonadherent cells were eluted with 50 mL of warm complete medium.

3H-racetyl cytotoxic assay. YAC-1 lymphoma cells maintained in culture were used as a target.19 Twofold serial dilution of nonadherent spleen cells were incubated in wells of 96-well round-bottomed microtiter plates with 3H-labeled YAC-1 cells (1.0 × 10⁴), giving five effector/target (E/T) ratios from 200:1 to 12.5:1. The total incubation volume was 0.2 mL. The plates were incubated at 37°C for four hours; the supernatant from each microwell was carefully harvested, and the radioactivity was measured by an auto-well gamma counter. Supernatants from microwells containing target cells alone were used as a control for the baseline release of ³HCr. Percent cytotoxicity was calculated as follows:

\[
\text{Cytotoxicity (%)} = \frac{\text{cpm of test group} - \text{cpm of baseline release}}{\text{total cpm in 10³ targets} - \text{cpm of baseline release}} \times 100
\]

RESULTS

Granules of mast cells in the auricle of some mutant rats were examined under the microscope. Only mast cells of DA rats with diluted coat color had giant granules. As shown in Figure 1, such mast cells were easily distinguishable from mast cells of the original DA strain rats. When blood smears were stained with Sudan Black, giant granules were observed in neutrophils of DA rats with diluted coat color. Prominent giant granules were also observed in pigment cells of the retina, iris and ciliary process (Fig 2).

All offspring that resulted from the cross between the DA rat with original agouti coat color and the mutant DA rat with diluted coat color (beige) had agouti coat color. The intercross among the resulting F₁ hybrids, and the backcross of F₁ hybrids to the mutant DA rat revealed that the beige mutation was autosomal recessive (Table 1). To confirm that normal and beige rats were congenic, skin pieces were exchanged between them. All eight grafts from normal rats onto the lateral thoracic wall of beige rats and all eight grafts from beige rats onto the lateral thoracic wall of normal rats were not rejected within the observation period (ie, 180 days).

Fig 1. Giant granules of mast cells in beige DA rats. (A) A mast cell in the auricle of a normal DA rat. (B) A mast cell in the auricle of a beige DA rat. Toluidine blue. (original magnification × 1,130).
cells in the ciliary process of a beige DA rat. Hematoxylin-eosin. (original magnification x 340).

Fig 2. Giant granules in pigment cells of a beige DA rats. (A) Pigment cells in the ciliary process of a normal DA rat. (B) Pigment cells in the ciliary process of a beige DA rat. (C) Pigment cells in the ciliary process of a non-beige DA rat.

Table 1. Segregation of Mutant Rats With Coat Color Dilution (Beige)

<table>
<thead>
<tr>
<th>Parent and Cross</th>
<th>Presumed Genotype of Parents</th>
<th>No. of Rats With Each Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agouti* x agouti</td>
<td>+/+ x +/+</td>
<td>203</td>
</tr>
<tr>
<td>Beige x beige</td>
<td>bg/bg x +/+</td>
<td>147</td>
</tr>
<tr>
<td>Agouti x beige</td>
<td>+/+ x bg/bg</td>
<td>115</td>
</tr>
<tr>
<td>F₁ x F₁</td>
<td>bg/± x bg/±</td>
<td>220</td>
</tr>
<tr>
<td>F₁ x beige</td>
<td>bg/± x bg/bg</td>
<td>63</td>
</tr>
</tbody>
</table>

*DA rats derived from the family, in which the beige mutation did not occur.

Table 2. Impaired Chemotaxis and Spontaneous Migration of Neutrophils in Beige Rats

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of Rats</th>
<th>Chemotactic Activity (A)</th>
<th>Spontaneous Migration (B)</th>
<th>Chemotactic Differential (A - B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/+</td>
<td>5</td>
<td>0.46 ± 0.05</td>
<td>0.16 ± 0.01</td>
<td>0.30 ± 0.04</td>
</tr>
<tr>
<td>bg/bg</td>
<td>5</td>
<td>0.04 ± 0.02*</td>
<td>0.02 ± 0.01*</td>
<td>0.02 ± 0.01†</td>
</tr>
</tbody>
</table>

*P < .01. †P < .05, when compared with the values observed in +/+ rats by t test.

Table 3. Prolonged Bleeding Time in Beige Rats and Its Normalization by Serotonin Injection

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Serotonin Injection</th>
<th>No. of Rats</th>
<th>Bleeding Time (min) (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/+</td>
<td>No</td>
<td>6</td>
<td>5.4 ± 1.0</td>
</tr>
<tr>
<td>bg/+</td>
<td>No</td>
<td>7</td>
<td>5.9 ± 0.6</td>
</tr>
<tr>
<td>bg/bg</td>
<td>No</td>
<td>7</td>
<td>&gt;15.0*</td>
</tr>
<tr>
<td>bg/bg</td>
<td>Yes</td>
<td>7</td>
<td>4.3 ± 0.4†</td>
</tr>
</tbody>
</table>

*In all bg/bg rats, cessation of bleeding was not observed within 15 minutes. P < .01, when compared with the values observed in +/+ or bg/+ rats by t test.
†P < .01, when compared with the value observed in nontreated bg/bg rats.

Table 4. Decrease in Blood Serotonin Concentration in Beige Rats

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of Rats</th>
<th>Blood Serotonin Concentration (µg/mL) (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/+</td>
<td>5</td>
<td>2.24 ± 0.06</td>
</tr>
<tr>
<td>bg/bg</td>
<td>5</td>
<td>0.22 ± 0.02*</td>
</tr>
</tbody>
</table>

*P < .01, when compared with the value observed in +/+ rats by t test.

Chemotactic activity of neutrophils is deficient in human CHS patients¹ and beige mice.⁷ We harvested neutrophils from the peritoneal cavity of the original and beige DA rats. As shown in Table 2, neutrophils of beige rats hardly showed either chemotaxis to zymosan-activated rat serum or spontaneous migration. As a result, "chemotactic differential" was significantly greater in normal rats than in beige rats.

Bleeding time was compared between the original and beige DA rats. Bleeding time was significantly prolonged in the mutant, and the prolongation was normalized by the injection of serotonin as reported in the case of beige mice (Table 3). This suggested that the prolonged bleeding time was due to the decrease in blood serotonin levels. In fact, the blood serotonin concentration in beige rats was about one tenth that of normal congenic rats (Table 4).

Spleen cell suspensions were prepared from the original and beige DA rats. The natural killer activity of spleen cells was significantly lower in the mutant DA rats than in the original DA rats (Fig 3).

DISCUSSION

An autosomal recessive mutation occurred in an inbred colony of the DA strain rat. The coat color was diluted, and...
apparent giant granules were observed in mast cells, neutrophils, and pigment cells of the eye. Either spontaneous migration or chemotaxis of neutrophils was deficient. The bleeding time was prolonged, and the blood serotonin level of the mutant was about one tenth that of the congenic normal rat. The injection of serotonin normalized the bleeding time of the mutant. Moreover, the natural killer activity of the mutant was significantly impaired. These pathological features are comparable to those of human CHS patients and beige mice.\(^1\)\(^5\)\(^6\)\(^29\)

Therefore, we considered this mutation of rats to be CHS, and designated it as beige (bg).

DA-bg/bg rats appeared healthy in conventional laboratory conditions, and were not significantly susceptible to bacterial and fungal infections. Since the genetic background of the bg mutation may influence to the magnitude of the susceptibility,\(^6\) we are now attempting to introduce the bg gene into other inbred strains of the rat by serial backcrosses.

Since DA-bg/bg rats are rather easy to breed and since rats are much larger than mice, they are probably useful for biochemical or molecular analysis of CHS. For the study of chemical carcinogenesis, rats are used more frequently than mice, at least in Japan.\(^17\) Therefore, DA-bg/bg rats will be a potentially useful model for studying the effect of natural killer activity on development of cancers\(^15\) after application of chemical carcinogens.

ACKNOWLEDGMENT

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