Granulocyte Function in the Chediak–Higashi Syndrome of Mice

By John I. Gallin, Joseph S. Bujak, Ethel Patten, and Sheldon M. Wolff

Granulocyte function was evaluated using peritoneal exudate granulocytes from a strain of beige mice with the Chediak–Higashi syndrome (CHS). Defective granulocyte chemotaxis of CHS cells (43% normal) was documented. There was no abnormality in the ability of CHS serum to function as a chemotactic stimulus. Phagocytosis of \(^{14}C\)-labeled Staphylococcus aureus by CHS granulocytes was normal. However, there was reduced bactericidal activity of \(S. \) aureus and group D streptococcus by CHS cells through 90 min of incubation. The bactericidal defect was most pronounced at early time periods and was related to impaired intracellular killing. These defects of CHS mice granulocytes were analogous to those reported in man and it is concluded that the CHS mouse is a most convenient and representative model of the CHS of man.

The Chediak–Higashi Syndrome (CHS) in man is a rare autosomal recessive disease characterized by partial oculocutaneous albinism, frequent pyogenic infections, neutropenia, and characteristic “giant” lysosomal granules present in all granule-containing cells.\(^1\) The disease has also been described in Aleutian mink,\(^2\) a strain of partial albino Hereford cattle,\(^3\) and recently in the beige mouse.\(^4,5\) Susceptibility to infection in man is associated with granulocytopenia,\(^6\) an abnormality of granulocyte chemotaxis,\(^7\) a delay in the killing of phagocytized bacteria, particularly staphylococci,\(^8\) and an abnormal distribution of enzymes in leukocyte lysosomes.\(^9\) When beige mice were recently found to have increased susceptibility to infection caused by Staphylococcus aureus, Streptococcus pneumoniae, and Candida albicans,\(^10\) studies to further characterize their leukocyte function were undertaken with the hope of establishing a convenient laboratory model of CHS.

MATERIALS AND METHODS

Animals

Control (black) mice (NIH stock C57 B6/JN) and CHS (beige) mice (NIH stock C57 B1/J Bg), both highly inbred strains\(^1\) were used throughout these studies. The animals weighed 20–25 g, were housed eight to twelve per cage, and fed Purina lab chow and water ad lib. Age- and sex-matched control and CHS animals were used for each experiment.
Serum

Blood was collected by transection of an axillary artery and pooled from several animals and allowed to clot (1 hr at 28°C). Serum was separated by centrifugation and stored at -7°C.

Granulocytes

Granulocytes were obtained from exudates 16 hr after intraperitoneal injection of 4 ml of sterile 10% sodium caseinate (Difco Laboratories, Detroit, Mich.) in isotonic saline. Immediately prior to harvesting the peritoneal exudates, 2 ml of heparinized (Upjohn Co., Kalamazoo, Mich.) saline (20 U/ml) was injected intraperitoneally. The animals were sacrificed by severing the spinal cord, the peritoneal cavity opened, and the exudate aspirated. Small amounts of contaminating erythrocytes were eliminated by one cycle of hypotonic saline lysis.7 The cells were washed twice in cold modified Hanks' solution.7 The cell yield by this method was 20-30 x 10^6 leukocytes per mouse with 80%-85% granulocytes and the rest mononuclear cells; there was no difference in the differential counts from normal or CHS animals. The characteristic large granules of CHS were present in leukocytes from the beige mice. Casein particles were ingested by both normal and CHS exudate cells, but there was no difference in the mean number of particles phagocytized by cells from each animal group (mean 4.7 ± 0.7 and 5.2 ± 0.6 casein particles per cell for CHS and normal mouse cells, respectively). Cells obtained from five normal or five CHS mice were pooled for each study.

Granulocyte Chemotaxis

Granulocyte chemotaxis was evaluated using a 51Cr radioassay recently described in detail.12,13 The peritoneal exudate leukocytes were labeled with 51Cr (Amersham/Searle Corporation, Arlington Heights, Ill.) and then adjusted to a concentration of 2.3 x 10^6 granulocytes/ml in Gey's balanced salt solution, pH 7.25, containing 2% bovine serum albumin, penicillin, and streptomycin (Gey's Medium, Microbiological Associates, Bethesda, Md.). There was no difference in the variable rates of 51Cr elution from normal or CHS leukocytes. The 51Cr-labeled cell suspension was placed in the upper compartment of a modified Boyden chamber,15 that was separated from the lower compartment by two 5-μm micropore filters (Millipore Corp., Bedford, Mass.). The chemotactic stimulus, serum alone, or serum activated with 30 g of endotoxin (Escherichia coli 0127:B8 lipopolysaccharide B, Difco Laboratories, Detroit, Mich.) as previously described,14 was placed in the lower compartment. After incubation in 100% humidity with 5% CO2 for 3 hr (unless indicated otherwise), the number of granulocytes migrating through the upper filter and into the lower filter has been shown to be proportional to the radioactivity incorporated into the lower of the two micropore filters in man16 and, for the present studies, was confirmed in CHS and normal mice. After adjusting for variable specific activity and incorporation of the 51Cr by the granulocytes, chemotaxis was expressed as corrected counts per minute lower filter (cor cpm LF). The chemotactic response in each individual experiment was taken as the average of four replicate chambers.

Phagocytic Studies

The phagocytic uptake of 14C-radiolabeled S. aureus was measured utilizing a previously reported method.4 Each experiment was run in triplicate and the percent uptake calculated as

\[
\text{(Average cell-associated cpm/Total cpm added)} \times 100.
\]

Bactericidal Assay

Bactericidal assays were performed in duplicate by the method of Hirsch and Strauss4 with modifications as previously described.4 Eighteen-hour cultures of NIH stock strains of a coagulase positive S. aureus, E. coli, and a group D streptococcus were used. Studies of the intracellular killing of bacteria were performed utilizing lysostaphin (Schwarz/Mann, Rockville, Md.) to kill extracellular but not intracellular bacteria.17 For this procedure 5 x 10^8 granulocytes in Hanks' balanced salt solution and 10% serum were preincubated at 37°C for 10 min and then 5 x 10^7 resting phase organisms were added (final volume 1.0 ml). The granulocyte–bacteria suspension was then tumbled in a roto-rack (Fisher Scientific Co., Rockville, Md.) for 20 min (12 tumbles per min) to allow phagocytic uptake to occur. One-tenth milliliter lysostaphin (final concentration 10 U/ml) was added, and with continued tumbling the number of viable organisms remaining in the lysostaphin-treated suspension was determined at 10,
60, and 120 min thereafter. Since lysostaphin destroyed all extracellular organisms by 10 min, the per
cent of viable intracellular organisms could be determined and compared to the total viable number
present in lysostaphin-free controls (intracellular plus extracellular). In addition, the rate of killing of in-
tracellular organisms could be subsequently determined.

Statistical Analysis
Standard error was used as an estimate of variance and the Student's t test was used to compare the
mean of different studies.

RESULTS

Granulocyte Chemotaxis

In every experiment migration of CHS mice granulocytes stimulated with chemotactic factor was significantly reduced compared with normal mice cells. Background levels of migration (chemotactic factor replaced by buffer), which measures passive migration,\(^8\) were consistently in the range of 30-50 cpm LF. Although there was a tendency for the background counts to be lower than normal in the CHS cells, the difference was not significant (\(p > 0.05\)).

As shown in Fig. 1, the defect of CHS mice granulocytes was noted throughout 4 hr of incubation of the chemotactic chambers with significant reduction in chemotaxis present by 90 min (\(p < 0.05\)). Although normal and CHS mice cells were less responsive to a heterologous chemotactic factor (endotoxin-activated human serum) than to a homologous chemotactic factor (Table 1), the defect in CHS granulocyte chemotaxis persisted (Table 1). There was no qualitative abnormality in the chemotactic factor resulting from endotoxin activation of CHS serum (Table 1).

Phagocytic Uptake and Bactericidal Activity

There was no difference in the phagocytic uptake by normal and CHS mice
granulocytes with the mean per cent cell-associated activity ± SE after 20 min of
tumbling in 10% serum at 37°C being 39.3 ± 0.9 and 39.2 ± 1.3, respectively. However, the ability of CHS leukocytes to kill \textit{S. aureus} was significantly less than
Table 1. Granulocyte Chemotaxis of CHS and Normal Mice to Different Stimuli

<table>
<thead>
<tr>
<th>Stimulus†</th>
<th>Normal Cells</th>
<th>CHS Cells</th>
<th>Per Cent of Normal</th>
<th>P †</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>185 ± 26</td>
<td>77 ± 3</td>
<td>43 ± 4</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>CHS</td>
<td>184 ± 14</td>
<td>77 ± 1</td>
<td>42 ± 3</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Human serum</td>
<td>63 ± 2</td>
<td>48 ± 1</td>
<td>76 ± 2</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

* Corrected counts per min lower filter (see text).
† Endotoxin-activated serum.
‡ Significance level of difference between CHS and normal.

controls when sampled at 30 min (p < 0.01, Table 2). Although still less effective than control cells at 90 min, the difference was no longer statistically significant. Studies using lysostaphin demonstrated that the abnormality in CHS granulocytes was related to a defect in the early intracellular killing of the S. aureus (Fig. 2). Similarly, the killing of group D streptococci by CHS leukocytes was significantly less than controls at 30 min (p < 0.05), but the difference was no longer significant at 90 min (Table 2). While the CHS leukocytes appeared to be less effective in killing E. coli as well, the results were not statistically significant (Table 2). The results of the phagocytic and bactericidal studies were independent of whether CHS or normal serum was used as a source of opsonins.

DISCUSSION

Studies on the mechanism of the recurrent pyogenic infections characteristic of the Chediak–Higashi syndrome have dealt primarily with abnormalities of leukocyte function. Defective leukocyte bactericidal capacity in the CHS has been reported in man6 and cattle19 but was not detected in the mink.19 Abnormal granulocyte chemotaxis has been documented in man7,20 and mink21 but not evaluated in cattle. Granulocytopenia, a characteristic finding in man,9 is not a feature of CHS in cattle,21 mink,22 or mice.5

The mechanism for the abnormal granulocyte functions in CHS is not known.

Table 2. Bactericidal Activity of Normal and CHS Mice Granulocytes

<table>
<thead>
<tr>
<th>Organism tested†</th>
<th>Bactericidal Activity‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 min</td>
</tr>
<tr>
<td>S. aureus</td>
<td></td>
</tr>
<tr>
<td>CHS (6)</td>
<td>184.3</td>
</tr>
<tr>
<td>normal (6)</td>
<td>115.5</td>
</tr>
<tr>
<td>Group D streptococcus</td>
<td></td>
</tr>
<tr>
<td>CHS (3)</td>
<td>240.0</td>
</tr>
<tr>
<td>normal (3)</td>
<td>99.3</td>
</tr>
<tr>
<td>E. coli</td>
<td></td>
</tr>
<tr>
<td>CHS (4)</td>
<td>212.5</td>
</tr>
<tr>
<td>normal (4)</td>
<td>129.2</td>
</tr>
</tbody>
</table>

* Number of experiments in parenthesis.
† Mean viable organisms expressed as per cent of the original inoculum.
‡ Paired sample student's t test.
The chemotactic defect may be related to an abnormality of the cell membrane or the presence in the cytoplasm of the large granules. As recently suggested, increased granulocyte rigidity could mechanically hinder the migration of CHS cells through the small pores of the micropore filter; magnification of the chemotactic defect with small pore size filters in man and mink supports this hypothesis. The abnormal bactericidal activity of granulocytes in CHS of man has been associated with impaired delivery of peroxidase from the “giant” lysosomes into the phagocytic vacuole. One might speculate that similar defects in the mouse could explain the results reported; however, such studies will need to be performed to substantiate such an hypothesis.

The current communication documents defective granulocyte chemotaxis and abnormal intracellular killing of coagulase positive S. aureus and group D streptococcus in CHS mice. The Chediak–Higashi mouse, therefore, is the first animal described with both the chemotactic and bactericidal granulocyte abnormalities reported in the human disease. Accordingly, the beige mouse is not only the most convenient but also the most representative laboratory model of the Chediak–Higashi syndrome in man.

REFERENCES

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21. Padgett GA: Personal communication
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