Granulocyte Chemotaxis in the Chediak-Higashi
Syndrome of Mink

By Robert A. Clark, Harry A. Kimball, and George A. Padgett

Studies of granulocyte chemotaxis were performed in mink with the Chediak-Higashi syndrome. In vivo migration of leukocytes to an inflammatory site was reduced in the affected animals. In vitro studies documented a consistent early impairment in the chemotactic response of Chediak-Higashi mink granulocytes. Serum from Chediak-Higashi mink generated normal amounts of chemotactic factors. The cellular defect in leukocyte chemotaxis in mink is comparable to that observed in humans with the Chediak-Higashi syndrome and may contribute to the increased susceptibility to infection in this disease.

The Chediak-Higashi Syndrome (CHS) is a rare disorder inherited as an autosomal recessive trait and characterized by partial oculocutaneous albinism and giant lysosomal granules in leukocytes and other cells. Following its description in humans, this disease was also found to occur in mink, cattle, and mice. In man, the most prominent clinical feature has been recurrent, severe, and often fatal pyogenic infections. An increased susceptibility to bacterial infections has also been noted in CHS mink, although the most frequent problem is Aleutian disease, a chronic viral infection.

Recent studies have demonstrated a cellular defect in granulocyte chemotaxis in humans with the Chediak-Higashi syndrome. The current report describes in vivo Rebuck skin window and in vitro Boyden chamber studies of leukocyte chemotaxis in CHS mink and documents a cellular chemotactic defect comparable to that observed in humans with CHS.

MATERIALS AND METHODS

Animals

The CHS mink had the aa genotype and sapphire coat color, while normal mink had either AA or Aa genotype and brown coat color. Animals were 6–8 mo of age, and, except as noted, none was infected with Aleutian disease virus or other pathogens. Serum globulin and blood urea nitrogen levels were normal.
Rebuck Skin Window Migration

The technique of Rebuck and Crowley was used to examine in vivo leukocyte migration. The magnitude of the cellular response at hourly intervals from 2 to 8 hr was graded from 0 to 4 in blinded fashion by two independent observers.

In Vitro Chemotaxis

Peritoneal exudates were collected in heparin (20 U/ml) 14 hr after i.p. infusion of 50 ml of 0.1% glycogen in Ringer’s lactate. Following hypotonic lysis of contaminating erythrocytes (0.2% NaCl for 20 sec), the cells were washed and resuspended (2.3 X 10^6 neutrophils/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.). The mean percentage of polymorphonuclear neutrophils (± SE) in these preparations was 83.5 ± 1.0 in the normal mink and 85.6 ± 0.8 in the CHS animals.

Details of the chemotaxis assay, adapted from Boyden, have been described. Fresh or freshly frozen (—70°C) mink serum (0.1 ml) was added to 0.1 ml of a 300 µg/ml solution of Escherichia coli endotoxin in 0.85% saline and mixed with 0.8 ml of gelatin-veronal-buffered saline supplemented with Mg^2+ (5 X 10^-4 M) and Ca^2+ (7 X 10^-5 M) cations. Chemotactic factor was generated by incubating this mixture at 37°C for 60 min, and residual complements was inactivated by heating at 56°C for an additional 30 min. In some experiments, freshly frozen guinea pig serum was used in place of mink serum.

In the chemotaxis chamber the compartment containing the chemotactic factor was separated from that containing the cells (1.8 X 10^6 neutrophils) by a 5 µm micropore filter (Millipore Corp., Bedford, Mass.). Following incubation for 1-4 hr at 37°C with 5% CO_2, the filters were stained, and the number of cells that had migrated to the lower surface of the filter counted in five to ten random fields. Chemotactic activity was expressed as the average number of cells per high power field (HPF). The chemotactic response in each individual experiment was taken as the average of four duplicate chamber values. The means of these responses for a number of separate experiments were compared using the standard two sample t test.

RESULTS

Rebuck Skin Window Migration

Skin window studies were performed on four normal and four CHS mink. There was a significant reduction in the number of migrating leukocytes in CHS mink (p < 0.01, Friedman rank sum test with partitioning of the Chi-square). The relative numbers of granulocytes and mononuclear cells in CHS animals were similar to normal, the majority of cells at these early time periods being polymorphonuclear leukocytes. Representative responses are shown in Fig. 1.

In Vitro Chemotaxis

In each experiment, cells from two or more CHS mink were studied concomitantly with cells from two or more normal animals. Background levels of migration (chemotactic factor replaced by buffer) were consistently in a range of 5–20 cells/HPF.

As summarized in Table 1, migration of CHS mink granulocytes was significantly reduced up through 3 hr of chamber incubation. The defect was greatest at earlier time periods, and, in fact, by 4 hr the CHS response was normal. Although mink cells were less responsive to a heterologous chemo-

tactic factor (endotoxin-activated guinea pig serum), the defect in CHS cell
Fig. 1. Rebuck skin windows. Representative fields from skin windows on normal mink (left) and CHS mink (right) at 2 hr (A, B), 3 hr (C, D), 4 hr (E, F), and 6 hr (G, H) are shown.
Table 1. Granulocyte Chemotaxis in CHS Mink

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Normal Cells</th>
<th>CHS Cells</th>
<th>Per Cent of Normal</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>14 ± 4† (4)</td>
<td>1 ± 0.7 (5)</td>
<td>7.1 &lt;0.02</td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>208 ± 9 (3)</td>
<td>38 ± 6 (3)</td>
<td>18.3 &lt;0.01</td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>303 ± 30 (6)</td>
<td>152 ± 17 (7)</td>
<td>50.2 &lt;0.01</td>
<td></td>
</tr>
<tr>
<td>3.0</td>
<td>379 ± 22 (10)</td>
<td>211 ± 18 (12)</td>
<td>55.7 &lt;0.01</td>
<td></td>
</tr>
<tr>
<td>4.0</td>
<td>362 ± 25 (5)</td>
<td>356 ± 22 (6)</td>
<td>98.3 &gt; 0.8</td>
<td></td>
</tr>
</tbody>
</table>

*Significance level of difference between CHS and normal.
†Mean ± SE, number of separate experiments in parenthesis.

chemotaxis persisted; at 3 hr mean chemotaxis in five experiments was 36 ± 8 for normal and 3 ± 1 for CHS cells (p < 0.01). As expected, decreasing the pore size of the filter resulted in less migration of both normal and CHS cells, but the magnitude of the CHS defect was considerably exaggerated. At 3 hr, pore sizes of 1.2, 3, and 5 μ resulted in CHS cell migration that was 18.8%, 26.5%, and 52.3% of normal, respectively.

Diminished viability in the CHS cells did not explain these results, since trypan blue exclusion in cells removed from the chemotaxis chamber at 3 hr indicated that 98% of both normal and CHS cells were viable. Passive motility and adhesiveness of CHS cells examined by the capillary tube migration technique were normal; mean migration (mm/hr) was 1.62 ± 0.28 for normal and 1.57 ± 0.25 for CHS cells (p > 0.5).

The chemotactic response of cells from CHS mink experimentally infected with Aleutian disease (AD) was less than that of cells from noninfected CHS mink (CHS with AD 126 ± 20, noninfected CHS 211 ± 18). In contrast, cells from normal mink with AD had normal chemotactic activity (normal with AD 430 ± 17, noninfected normal 379 ± 22).

Endotoxin activation of CHS mink serum generated normal amounts of chemotactic factors (Table 2). The presence of AD had no significant effect on the serum of either normal or CHS mink.

**DISCUSSION**

Studies of host resistance in the Chediak-Higashi syndrome have dealt primarily with leukocyte regulation and function. A number of abnormalities have been documented including: granulocytopenia,14 decreased marrow granulocyte mobilization,5 decreased postphagocytic degranulation,5,16 defective leukocyte bactericidal capacity,16,17 reduced levels and abnormal distribution of lysosomal enzymes,18 and a cellular defect in granulocyte chemotaxis in humans with CHS.8

In the current report, the in vivo skin window studies document a decreased accumulation of inflammatory cells. This cannot be attributed to neutropenia, since CHS mink, unlike humans with this disease, have normal neutrophil counts. The in vitro studies of chemotaxis demonstrate a consistent reduction in the migration of CHS mink neutrophils. This defect was most profound
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Table 2. Chemotactic Activity of Endotoxin-Activated Serum From Normal and CHS Mink

<table>
<thead>
<tr>
<th>Serum</th>
<th>Chemotactic Activity</th>
<th>$p^*$</th>
</tr>
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<tbody>
<tr>
<td>Normal</td>
<td>358 ± 26† (5)</td>
<td>—</td>
</tr>
<tr>
<td>CHS</td>
<td>386 ± 19 (6)</td>
<td>&gt; 0.3</td>
</tr>
<tr>
<td>Normal with AD†</td>
<td>319 ± 25 (4)</td>
<td>&gt; 0.3</td>
</tr>
<tr>
<td>CHS with AD</td>
<td>321 ± 37 (6)</td>
<td>&gt; 0.4</td>
</tr>
</tbody>
</table>

*Significance level of difference from normal.
†Mean ± SE, number of separate experiments in parenthesis.
‡Aleutian disease, see text.

at early incubation times and was observed with two different chemotactic stimuli. No abnormalities of passive motility or viability were found to explain these results. Aleutian disease, a slow virus infection characterized by hypergammaglobulinemia, lymphoid infiltration, and progressive renal failure, occurs commonly in CHS mink and occasionally in normal mink. Our data suggest that CHS mink with AD may have an even greater chemotactic defect. The finding that CHS mink serum generated normal amounts of chemotactic factors is consistent with the lack of any known abnormalities of the complement system in these animals and emphasizes the cellular nature of the CHS chemotactic defect.

Attempts were made to study in vitro chemotaxis in CHS and normal cattle, but since the cow leukocytes do not adhere well to the micropore filter reliable counts of migrating cells could not be obtained.

The mechanism for the diminished chemotactic response in CHS leukocytes is not understood. A failure in recognition of a specific stimulus seems unlikely, since the defect in humans was observed with three clearly distinct chemotactic factors and in mink was seen with either mink or guinea pig serum as a complement source. CHS leukocytes may be less deformable than normal cells, due to a membrane abnormality or to the presence in the cytoplasm of giant lysosomal granules that may reach 4 μ in diameter. An increase in rigidity could mechanically hinder the CHS leukocytes in passing through small pores in the filter or in migrating from the blood to a site of infection. Magnification of the defect with smaller pore size filters seems consistent with such a hypothesis. Alternatively, a biochemical abnormality in the presumably complex series of events leading from stimulus recognition to migration could be implicated.

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REFERENCES

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