Defective Mononuclear Leukocyte Chemotaxis in the Chediak-Higashi Syndrome of Humans, Mink, and Cattle

By John I. Gallin, Joan A. Klimerman, George A. Padgett, and Sheldon M. Wolff

Chemotaxis of mononuclear leukocytes from humans, mink, and cattle was evaluated in vitro using a morphologic Boyden chamber technique and a new $^{31}$Cr-labeled mononuclear radioassay with a double micropore filter system. Significantly decreased mononuclear leukocyte chemotactic responses were noted when human, mink, or cattle Chediak-Higashi cells were tested using autologous serum or endotoxin-activated autologous serum. A similar Chediak-Higashi mononuclear leukocyte defect was noted in humans when kallikrein or dialyzable transfer factor were used as the chemotactic stimulus. Studies using smaller pore filters in the chemotactic chamber exaggerated the chemotactic defect. Serum from Chediak-Higashi subjects had normal chemotactic activity. Additional studies on the spontaneous (random) locomotion of Chediak-Higashi mononuclear leukocytes revealed normal results when a capillary tube assay system was used, but abnormal results were obtained when a Boyden chamber micropore filter assay was used, demonstrating fundamental differences in these two assays of random locomotion. It is clear from these studies that defective mononuclear leukocyte chemotaxis is another feature of the impaired host defenses in the Chediak-Higashi syndrome that may contribute to the marked susceptibility to pyogenic infections so characteristic of this disease.

THE CHEDIAK-HIGASHI syndrome (CHS) is a rare disease characterized by partial ocucutaneous albinism, neutropenia, recurrent pyogenic infections, giant lysosomes in all lysosomal-containing cells, and an autosomal recessive pattern of inheritance. A similar disease has been described in mink, cattle, mice, and whales. Extensive studies of host defenses in this disease have failed to reveal any abnormalities of humoral components such as ability to make antibody, or complement. However, studies of leukocyte function have documented defective granulocyte chemotaxis in humans, mink, and mice with CHS and impaired (delayed) intracellular killing of phagocytized bacteria by neutrophils from CHS humans and mice. The following studies using in vitro Boyden chamber techniques demonstrate abnormal directed chemotaxis and random (spontaneous) mononuclear cell locomotion in the CHS of man, mink, and cattle.

MATERIALS AND METHODS

Human Subjects

Healthy laboratory personnel served as normal controls. Two brothers with CHS, who have been previously described in detail, were studied at times when they were not infected.

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Mink

The CHS mink had an aa genotype and sapphire coat color, while normal mink had either AA or Aa genotype and brown or black coat color. Animals were 6-8 mo old, and none were infected with Aleutian disease or other pathogens. The mink were from the College of Veterinary Medicine, Washington State University, Pullman, Wa.

Cattle

The CHS cattle were from the experimental CHS Hereford herd maintained by Washington State University. The normal cattle were from registered Hereford stock. All of the cattle were approximately 8 mo old and weighed 300-400 lb.

Blood

For each species studied, peripheral venous blood was anticoagulated with heparin (Upjohn Co., Kalamazoo, Mich.; 20 μ/ml). Mononuclear leukocytes were separated from whole blood by the Hypaque-Ficoll technique. This technique routinely resulted in greater than 95% pure mononuclear cells. The differential cell counts of the mononuclear cell preparations were about 80% lymphocytes and 20% monocytes for each species; there were no differences between CHS and normal subjects. Sera were obtained as previously described.

Assay of Directed Locomotion (Chemotaxis)

Mononuclear leukocyte chemotaxis was measured using the morphologic assay previously described by Snyderman et al. employing a 5-μ micropore filter (Nucleopore, Wallabs, Inc., San Rafael, Calif.). Chemotaxis was expressed as the mean number of cells per high-power field migrating to the lower surface of the filter. Four replicate chambers were used for each determination.

For some studies of mononuclear leukocyte locomotion, a modification of a 51Cr radioassay of neutrophil chemotaxis was used. For this assay a Nucleopore filter with the dull surface up was placed on top of a 8.0-μ micropore filter (Sartorius Membranfilter, Gottingen, West Germany). Hypaque-Ficoll mononuclear leukocytes were labeled with 51Cr (Amersham/Searle, Arlington Heights, Ill.) using an identical method to that described for neutrophils. After labeling, the cells were washed three times in modified Hanks' medium and then suspended in Gey's balanced salt solution (Microbiologic Associates, Bethesda, Md.) at a density of 3.0 x 10⁶ cells per ml. The labeling-washing procedure was associated with a 35%-45% cell loss, and studies of the radioactivity of glass-adherent and non-glass-adherent cells showed no differences between cell losses in CHS and normals. There was no difference in the uptake of 51Cr by CHS or normal mononuclear cells, and there was no significant elution of the isotope from CHS or normal cells during the duration of the study. The 51Cr did not modify normal or CHS mononuclear cell migration as assessed with the morphologic technique. For the radioassay, the 51Cr-labeled mononuclear leukocytes were shown morphologically to traverse the upper filter and migrate into the lower filter. Following incubation for 3 hr, the lower filters were removed, rinsed in saline, and then counted in a gamma counter. After adjusting for day-to-day variability in the uptake of 51Cr by the mononuclear leukocytes, chemotaxis was expressed as corrected counts per minute lower filter (cor cpm LF) as previously described. With this assay, a linear dose-mononuclear leukocyte chemotactic response was demonstrated for different concentrations of serum (0.5-50 μl) added to the lower compartment of the chemotactic chambers. In addition, a linear correlation was demonstrated for the number of cells added to the chemotactic chamber and the chemotactic response. These studies were analogous to paired studies using the morphologic single-chamber method and were similar to the published data obtained with the 51Cr neutrophil assay.

Chemotactic Factors

The chemotactic activity of endotoxin (E. coli: 0127:B8 lipopolysaccharide B, Difco Labs., Detroit, Mich.)-activated whole serum was determined using published techniques. For some studies, human dialyzable transfer factor and kallikrein, both of which have chemotactic activity for human mononuclear leukocytes, were prepared as previously reported.
Assay of Spontaneous Random Locomotion

Spontaneous motility was assessed by two techniques. One method employed the modified Boyden chamber in which mononuclear leukocyte (3.0 x 10^6 cells per ml) migration through 5.0-μm Nucleopore filters was assessed morphologically using Hanks' balanced salt solution as the chemotactic stimulus. The other technique was our modification of Bryant et al.'s capillary tube assay, in which mononuclear leukocytes (3.0 x 10^6/ml), suspended in autologous serum, were centrifuged in microhematocrit tubes, positioned vertically, and incubated at 37°C for 3 hr. Random migration was expressed as the distance from the front of migration to the base of the cell pellet.

Statistics

The means of responses for a number of separate experiments were compared using the Student's t test. Standard error was used throughout as an estimate of variance.

RESULTS

Studies of CHS Mononuclear Leukocyte Chemotaxis

Initial experiments were performed using the morphologic assay. In each experiment in man, mink, or cattle, the chemotactic response of CHS mononuclear leukocytes was significantly reduced compared with that of normals (Fig. 1). It is noteworthy that mink and cattle mononuclear leukocytes migrated less well than human cells. The CHS mononuclear leukocyte chemotactic response to endotoxin-activated serum was significantly reduced to 47% of normal for humans, 54% of normal for mice, and 38% of normal for cattle (p < 0.05 for each species). There were no defects in the ability of nonactivated or endotoxin-activated CHS sera to attract cells, demonstrating that the abnormal mononuclear leukocyte chemotactic response was secondary to a cellular and not a serum defect.
Table 1. Chemotactic Response of Normal and CHS Human Mononuclear Leukocytes to Different Stimuli

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Normal Leukocytes</th>
<th>CHS Leukocytes</th>
<th>Per cent of Normal</th>
<th>p†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonactivated normal serum</td>
<td>246 ± 38 (4)†</td>
<td>113 ± 15 (3)</td>
<td>46</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Endotoxin-activated normal serum</td>
<td>816 ± 72 (5)</td>
<td>346 ± 28 (4)</td>
<td>42</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Dialyzable transfer factor</td>
<td>243 ± 14 (3)</td>
<td>75 ± 12 (2)</td>
<td>31</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Kallikrein-plasminogen activator mixture</td>
<td>495 ± 41 (4)</td>
<td>232 ± 8 (2)</td>
<td>47</td>
<td>&lt; 0.02</td>
</tr>
</tbody>
</table>

*Cells per hpf, morphologic assay.
†Significance level of difference from normal.
†Number of different experiments in parentheses.

Kinetics of Mononuclear Leukocyte Chemotaxis

The results shown in Fig. 1 and Table 1 were obtained using a 90-min-incubation period with the morphologic chemotactic assay. In additional studies, the time course of mononuclear leukocyte migration was varied from 30 min to 3 hr (Fig. 2). It was noted that, using the morphologic assay, the response of CHS cells was significantly defective throughout 3 hr in humans (p < 0.01), 2 hr in mink (p < 0.05), and 90 min in cattle (p < 0.05). The abnormality of CHS cells from mink and cattle was not detectable after 3 hr because normal cells fell off the bottom surface of the micropore filter and were detected in the lower compartment of the chemotactic chambers.

Defective mononuclear cell chemotactic responses were also demonstrated in humans and mink using a $^{51}$Cr radioassay (Fig. 3). It is apparent that, with this assay, CHS human and mink mononuclear cell chemotactic responses were abnormal throughout 3 hr of incubation. Cattle were not studied with the radioassay. Using the double micropore filter system, the mononuclear cells do not fall off the lower surface of the filter but rather are trapped in the lower filter. Therefore, the decreased normal chemotactic response at the later times noted with the morphologic assay (Fig. 2) were not present.
**Chemotactic Response to Different Stimuli**

The ability of normal and CHS human mononuclear cells to respond to the four different chemotactic stimuli, nonactivated serum, endotoxin-activated serum, dialyzable transfer factor, and a kallikrein-plasminogen activator mixture, was evaluated using the morphologic assay (Table 1). For each stimulus, the chemotactic response of CHS mononuclear cells was significantly abnormal, ranging from 31% of normal with dialyzable transfer factor to 47% of normal with a kallikrein-plasminogen activator mixture.

**Studies of Random (Spontaneous) Locomotion**

As shown in Table 2, there was no difference between CHS and normal cells in the spontaneous migration of mononuclear leukocytes in the capillary tube. In contrast, assessment of spontaneous locomotion using the morphologic Boyden chamber assay demonstrated a significant \( p < 0.02 \) abnormality in CHS cells (31% of normal, Table 2).**

**Effect of Micropore Filter Pore Size on the Chemotactic Response**

The effect of decreasing the pore size of the Nucleopore filters on normal and CHS human mononuclear cell chemotactic responses to endotoxin-activated normal human serum was studied using the morphologic assay and 2-\( \mu \) Nucleo-

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**Table 2. Spontaneous (Random) Locomotion of Normal and CHS Human Mononuclear Leukocytes**

<table>
<thead>
<tr>
<th>Cell Source</th>
<th>Capillary Tube (mm migrated ( \times 10^{-1} ))</th>
<th>Boyden Chamber (cells/hpf)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>( 2.08 \pm 0.27 ) (6)*</td>
<td>( 45 \pm 7 ) (5)</td>
</tr>
<tr>
<td>CHS</td>
<td>( 1.86 \pm 0.12 ) (6)†</td>
<td>( 14 \pm 6 ) (6)‡</td>
</tr>
</tbody>
</table>

*Mean \( \pm \) standard error; number of determinations in parentheses.
†No significant difference between CHS and normal \( (p > 0.05) \).
‡Difference between CHS and normal is significant \( (p < 0.02) \).
pore filters. With 2-μ filters, normal mononuclear leukocytes had a chemotactic response of 120 ± 18 cells per hpf, while CHS mononuclear leukocytes had a response of 26 ± 4 cells per hpf. Thus, with smaller-pore filters, the CHS mononuclear cell chemotactic defect was exaggerated (22% of normal with 2-μ filters compared with 42% of normal using 5-μ filters).

DISCUSSION

The demonstration of impaired mononuclear leukocyte locomotion in CHS leukocytes obtained from three different species adds another defect to the list of abnormal host defenses already documented in this disease. The other abnormalities include granulocytopenia,1 decreased granulocyte mobilization,20 failure of phagocytic degranulation,10 defective leukocyte bactericidal capacity,9,10 reduced levels and abnormal distribution of lysosomal enzymes,21 and a cellular defect in granulocyte chemotaxis.7,9 The finding that CHS serum generated qualitatively normal chemotactic factors agrees with the findings of others,7 is consistent with the lack of any known abnormalities of the complement system in this disease, and emphasizes the cellular nature of the CHS defect. The association of defective mononuclear cell chemotaxis in a disease with a previously described granulocyte defect7,9 of humans, mink, and mice with CHS suggests that there is a generalized abnormality of all motile cells rather than a defect restricted to a single cell line.

Locomotion of leukocytes has been separated into two distinct categories: random (spontaneous) and directed (chemotactic) motility. The latter probably represents a perturbation of the random process.22 In an effort to describe the nature of the defective mononuclear cell locomotion in CHS, both processes were studied. Two methods of assessment of random motility (capillary tube and Boyden chamber assay) were evaluated because of growing evidence that these methods do not measure identical parameters.23 The Boyden chamber-micropore filter technique requires cell adherence to the filter and movement through the small pores of the micropore filters, whereas the capillary tube technique measures cell movement up the capillary tube without the added requirement of adherence to filters or movement through narrow pores. The spontaneous locomotion of CHS leukocytes was normal with the capillary tube method but abnormal with the Boyden chamber technique, emphasizing differences in the parameters measured with these two methods.

The defective CHS mononuclear leukocyte chemotaxis observed using the morphologic in vitro assay was confirmed with a new 51Cr radioassay. The latter assay was adopted from a similar 51Cr isotope technique developed for granulocyte chemotaxis13,14 and proved a simple, reliable, rapid, and objective assay of mononuclear leukocyte locomotion. The only major limitation of the radioassay of mononuclear leukocyte chemotaxis was decreased cell yield (35%-45% cell loss after labeling cells with 51Cr); in the future the use of miniaturized chemotactic chambers should minimize this disadvantage. With the radioassay, morphologic distinction of migrating mononuclear cells is not possible; however, we have found that non-glass-adherent lymphocytes do not migrate with caseinate or serum as the chemotactic stimulus, and, morphologically, only macrophages enter the lower filter (Gallin, unpublished observation).
The mechanism for the abnormal leukocyte locomotion in CHS is unknown. The CHS granulocyte and mononuclear leukocyte chemotactic defects were noted for a variety of different chemotactic factors, so it is unlikely there is a specific abnormality in recognition of the chemotactic factor. In a previous study of defective granulocyte chemotaxis in CHS, it was speculated that the defect was related to decreased cell distensibility secondary to the 4-μm intracellular giant lysosome inclusions. Support for this concept was the observation that decreasing the pore size of the filter exaggerated the granulocyte defect. Similar results are reported in the current study with CHS mononuclear leukocytes which also contain the large granules. Decreased distensibility of CHS leukocytes would also explain the observation of normal capillary tube but abnormal Boyden chamber assays of spontaneous movement, since, in the capillary tube assay, cells do not have to squeeze through narrow pores. Recent studies of normal leukocyte locomotion have demonstrated an association between exposure of cells to chemotactic factors and calcium release, microtubule assembly, increased rate of aerobic glycolysis, and increased activity of the hexose monophosphate shunt. We were not able to document any abnormality in the release of 45Ca from CHS neutrophils or mononuclear leukocytes on exposure to chemotactic factors (Gallin, unpublished observation). However, the hexose monophosphate shunt activity is known to be abnormally accelerated in CHS leukocytes. Evaluation of this and other biochemical pathways in CHS cells exposed to chemotactic factors awaits additional study.

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