Functional and Metabolic Studies of Polymorphonuclear Leukocytes in the Congenital Pelger-Huet Anomaly

By Christine A. Johnson, David A. Bass, Alberto A. Trillo, Melinda S. Snyder, and Lawrence R. DeChatelet

Polymorphonuclear leukocytes (PMNL) from two individuals with congenital Pelger-Huet anomaly (PHA) were examined to determine whether functional or metabolic defects accompanied the known morphological abnormality. No abnormalities of the PHA cells, as compared to normal control cells, were found when tested for quantitative leukocyte enzyme activities, nitroblue tetrazolium reduction, hexose monophosphate shunt activity, superoxide production, generation of chemiluminescence, or iodination. The PHA cells, as compared to normal PMNL, demonstrated normal chemotaxis and random migration, as well as bactericidal activity.

CONGENITAL Pelger-Huet anomaly (PHA), which is inherited as a simple autosomal dominant trait, affects approximately 1 in 6000 individuals in the United States. The heterozygous condition is characterized by the finding, in peripheral blood, of a predominance of two-lobed polymorphonuclear leukocytes (PMNL) having the typical pince-nez appearance. Awareness of the disorder is important in order to prevent confusion with a severe “shift-to-the-left” of the differential blood smear. It is generally felt that this syndrome is not associated with an increased incidence of severe infections, although an affliction by localized infections, such as minor furunculosis, has been cited.

In this article, PMNL from two related individuals with congenital Pelger-Huet anomaly were studied to determine specific enzymology, metabolism, function, and morphology of affected leukocytes in vitro.

MATERIALS AND METHODS

Case Report

M.G., a 28-yr-old white female of English-French-Canadian ancestry, was found to have the typical morphological changes (as determined by light and electron microscopy) seen in PMNL from individuals heterozygous for PHA. Her 4-yr-old daughter's cells were similarly altered. Both were clinically well; neither had a history of recurrent severe infections or other hematologic abnormalities. No other affected family members were identified.

Isolation of Leukocytes for Metabolic and Enzymatic Studies

Human PMNL were isolated as previously described. Viability was >95% as determined by trypan blue dye, and more than 80% of the isolated cells were PMNL.

Enzymatic and Metabolic Studies

All patient studies were compared to those of control neutrophils from normal donors run at the same time in exact parallel. Quantitative leukocyte enzyme activities were determined using standard methods. Nitroblue tetrazolium (NBT) reduction was measured in resting cells and in cells challenged with latex particles, as described by Baehner and Nathan. Results were expressed as OD/40 min/2.5 x 10^6 cells. Hexose monophosphate shunt activity was determined in resting cells and in cells challenged with zymosan particles by measuring the conversion of glucose-1-14C to 14CO2. Results were expressed as counts per minute (cpm) in 14CO2/hr/5 x 10^6 cells. Superoxide production was measured by the method of Babior. Reactions employed resting PMNL and PMNL challenged with either unopsonized zymosan or zymosan opsonized with normal pooled human serum in the presence and absence of 50 μg of superoxide dismutase. Results were expressed as change in absorbance at 550 nm (ΔOD/30 min/5 x 10^6 cells).

Generation of chemiluminescence during phagocytosis was determined at 10-min intervals using a Beckman LSC-100 liquid scintillation spectrometer operated in out-of-coincidence mode as described by Johnston et al. Iodination was determined under both resting and phagocytizing conditions by measuring cellular incorporation of 125I (New England Nuclear Corp., Boston, Mass.) into trichloroacetic acid insoluble material as previously described. Results were expressed as cpm/5 x 10^6 cells/30 min.

Leukocyte Function Studies

Chemotaxis and random migration of PMNL were measured by a modification of the chemotaxis-under-agarose technique described by Nelson and modified by Bass. Zymosan-activated serum served as the chemotactic agent; normal serum was employed in the control well to assess random migration. For the bactericidal assay, a modification of the method of Maaløe was employed The test organism was a strain of Proteus mirabilis previously shown to be appropriate in this assay system.

RESULTS

Enzymatic and Metabolic Studies

The activities of a number of leukocytic enzymes, both membrane-associated (Mg2+ ATPase and alkaline phosphatase) and granule-associated (acid phosphatase, lysozyme, β-glucuronidase, and myeloperoxidase) were compared in control and PHA cells (Table 1). The specific activities were all within the normal range in the PHA cells, with the exception of alkaline phosphatase, which was slightly elevated. The activity of this enzyme was assayed over a pH range of 8.5–11.0 with a different patient sample and...
Table 1. Specific Activities of Various Enzymes in Pelger-Huet and Control Cells

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Control</th>
<th>Control</th>
<th>PHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg$^{2+}$-ATPase</td>
<td>0.153</td>
<td>0.179</td>
<td>0.168</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>1.45</td>
<td>0.93</td>
<td>2.91</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>20.5</td>
<td>13.4</td>
<td>14.0</td>
</tr>
<tr>
<td>$\beta$-glucuronidase</td>
<td>84.6</td>
<td>50.0</td>
<td>56.4</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>0.88</td>
<td>1.10</td>
<td>1.20</td>
</tr>
<tr>
<td>Myeloperoxidase</td>
<td>0.84</td>
<td>0.51</td>
<td>0.76</td>
</tr>
</tbody>
</table>

All values represent the mean of triplicate determinations run at different levels of enzyme concentration. Specific activities are defined as follows: Mg$^{2+}$-ATPase—$\mu$ mole phosphate released/hr/mg; alkaline and acid phosphatase—$\mu$ mole $p$-nitrophenol released/hr/mg; $\beta$-glucuronidase—$\mu$ mole phenolphthalein released/hr/mg; lysozyme—$\Delta$OD/min/mg; myeloperoxidase—$\mu$ mole o-dianisidine reacted/min/mg.

Fig. 1. Leukocyte alkaline phosphatase activity.

compared to cells from a normal control. The specific activities of the two samples were comparable over the entire pH range (Fig. 1) and were consistent with previously reported values. Thus, there appears to be no difference in any of the enzymes examined between PHA and control cells. Nitroblue tetrazolium reduction by PHA leukocytes at rest and after challenge with latex particles was examined on two separate occasions over a year apart (Table 2). In the first experiment, the PHA cells appeared to show increased reduction, but on reexamination, the PHA and control results were similar. Hexose monophosphate shunt activity in resting and phagocytizing Pelger-Huet cells was normal as compared to control (Table 3). Both control and Pelger-Huet cells exhibited very little superoxide production at rest or when exposed to unopsonized zymosan. Upon challenge with opsonized zymosan, however, the PHA cells showed a generation of superoxide anion equivalent to that exhibited by normal control cells (Table 4). PHA cells generated chemiluminescence during phagocytosis, which was at least equivalent to that observed with leukocytes from normal controls (Fig. 2). A second experiment performed 1 yr later, yielded similar results (data not shown). Iodination by PHA leukocytes was normal (Table 5).

Functional Studies

Random motility of PHA cells was equivalent to that of normal controls (Table 6). PHA and control cells responded to the chemotactic stimulus in zymosan-activated serum (presumably C5a) with a migration of 204% and 231% of random migration, respectively; this difference was not statistically significant. Absolute chemotactic migration of PHA and control cells were identical (Table 6). After 2 hr, both PHA and control cells killed over 95% of bacteria at an initial bacteria:cell ratio of 5:1 (Table 7). The rates of killing by PHA and control cells were identical.

DISCUSSION

The Pelger-Huet anomaly has been felt to be a morphological disorder associated with minimal to no

Table 2. Quantitative Nitroblue Tetrazolium Reduction of Control and Pelger-Huet Leukocytes

<table>
<thead>
<tr>
<th></th>
<th>Control cells</th>
<th>Pelger-Huet cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control cells</td>
<td>0.130</td>
<td>0.259</td>
</tr>
<tr>
<td>Pelger-Huet cells</td>
<td>0.205</td>
<td>0.340</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control cells</td>
<td>0.149</td>
<td>0.435</td>
</tr>
<tr>
<td>Control cells</td>
<td>0.162</td>
<td>0.593</td>
</tr>
<tr>
<td>Pelger-Huet cells</td>
<td>0.150</td>
<td>0.478</td>
</tr>
</tbody>
</table>

The two experiments were performed on cells from the same patient over 1 yr apart. Each value represents the mean of closely agreeing triplicate determinations in each experiment.

Table 3. Hexose Monophosphate Shunt Activity of Control and Pelger-Huet Cells: Oxidation of Glucose-1$^{14}$C

<table>
<thead>
<tr>
<th></th>
<th>Control cells</th>
<th>Pelger-Huet cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting</td>
<td>1,380</td>
<td>2,154</td>
</tr>
<tr>
<td>Phagocytizing</td>
<td>38,285</td>
<td>37,332</td>
</tr>
</tbody>
</table>

Each value represents the mean of closely agreeing triplicate determinations.

Table 4. Superoxide Production by Control and Pelger-Huet Leukocytes

<table>
<thead>
<tr>
<th></th>
<th>Control cells</th>
<th>Pelger-Huet cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zymosan</td>
<td>0.012</td>
<td>0.016</td>
</tr>
<tr>
<td>Opsonized Zymosan</td>
<td>0.256</td>
<td>0.264</td>
</tr>
</tbody>
</table>

Table 5. Lymphocyte chemotaxis.

Each value represents the mean of closely agreeing triplicate determinations.
increase in propensity to infection. The homozygous form has been reported to be associated with mortality in the early years of life. The deaths may have been due to infections, but this was not clearly documented.\textsuperscript{13} Compromise of a single component of the functional armamentarium of neutrophils may cause a limited increase in susceptibility to infection. For example, myeloperoxidase deficiency results in a moderate impairment of neutrophil bactericidal activity by preventing multiple peroxidase-hydrogen peroxide-halide cidal mechanisms; yet such patients may have no obvious inability to handle infection and may present with no more than persistent acne.\textsuperscript{14,15} To our knowledge, neutrophils of patients with PHA have not been examined for such specific biochemical or metabolic defects.

In this study, oxidative metabolic responses of PHA neutrophils were found to be unimpaired. Thus, superoxide production, chemiluminescence, protein iodination, hexose monophosphate shunt activity, and reduction of nitroblue tetrazolium were all appropriately stimulated by phagocytosis, with a quantitative response equal to that of normal neutrophils. Quantitative enzyme assays were normal, in agreement with previous studies.\textsuperscript{1,17} Although degranulation was not studied specifically, the ability to ingest and kill bacteria was equal to that of normal leukocytes. Further, the finding of normal iodination by the PHA cells strongly suggests that degranulation of myeloperoxidase, at least, was normal. Normal bactericidal activity as well as NBT reduction has been previously reported in cells from a patient with PHA, although no data were presented.\textsuperscript{2}

Random migration and chemotaxis were also identical to those of normal cells. The chemotaxis-under-agarose technique examines the ability of PMNL to migrate on a surface and is a sensitive technique for studying alterations of migration due to metabolic or membrane changes. For example, even usual methods of leukocyte collection alter membrane morphology and subsequent migration in this assay. However, the method does not examine the ability of leukocytes to diapedese through a small opening, as is examined in the Boyden technique when membranes of small pore size are used. Although PHA cells migrate normally on a flat surface, they have recently been reported by Park et al.\textsuperscript{1} to be less able to diapedese through a small pore membrane. This finding is in agreement with Rebuck's observation that fewer PHA leukocytes than normal cells accumulate in a skin window in response to inflammation in vivo.\textsuperscript{18} It was speculated that such decreased migration might be due to the mechanical

\begin{table}
\centering
\caption{Iodination in Control and Pelger-Huët Leukocytes (Fixation of $^{125}$I to Ingested Particle)}
\begin{tabular}{ll}
\hline
 & \textbf{$^{125}$I-fixed cpm/30 min/} \\
 & \textbf{5 x 10$^6$ PMNL} \\
\hline
Control (without cells) & 2,077 \\
Control cells, resting & 2,224 \\
Control cells, phagocytizing & 81,698 \\
Pelger-Huët cells, resting & 2,449 \\
Pelger-Huët cells, phagocytizing & 106,244 \\
\hline
\end{tabular}
\end{table}

\begin{table}
\centering
\caption{Bactericidal Activity of Control and Pelger-Huët Leukocytes (Proteus 5.18 x 10$^7$/ml)}
\begin{tabular}{llll}
\hline
 & \textbf{Viable Bacteria at} & \textbf{60 min} & \textbf{120 min} \\
\hline
Serum alone & 3.07 x 10$^8$ & 9.96 x 10$^8$ \\
Control cells & 0.54 x 10$^8$ & 0.35 x 10$^8$ \\
Pelger-Huët cells & 0.40 x 10$^8$ & 0.36 x 10$^8$ \\
\hline
\end{tabular}
\end{table}
STUDIES IN PELGER-HUET ANOMALY

hindrance by the large unsegmented nucleus of the cells.

Thus, the abnormalities described with the Pelger-Huet anomaly appear to be limited to morphological changes and an impaired ability to migrate through small openings. The biochemical, metabolic, phagocytic, and bactericidal activities of the cells, as well as their random motility and response to a chemotactic stimulus, are all equivalent to normal neutrophil leukocytes.

REFERENCES

5. Beck WS: The control of leukocyte glycolysis. University of California School of Medicine Atomic Energy Project (Los Angeles), 27 November 1957
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CA Johnson, DA Bass, AA Trillo, MS Snyder and LR DeChatelet