Expression of the Chediak-Higashi Lysosomal Abnormality in Human Peripheral Blood Lymphocyte Subpopulations

By Carlo E. Grossi, William M. Crist, Toru Abo, Andrea Velardi, and Max D. Cooper

Fusion of lysosomes to form a giant cytoplasmic inclusion is a major abnormality expressed by multiple hematopoietic and non-hematopoietic cell types in Chediak-Higashi (C-H) patients. In this study, the extent of involvement of lymphoid cell subpopulations was defined. Purified populations of B cells, natural killer (NK) cells, and helper T cells were obtained from two C-H patients and normal controls by immunofluorescence staining of their blood mononuclear cells with the monoclonal antibodies HB-2, Leu-7, or Leu-3 followed by fluorescence-activated cell sorting. Cytochemical and ultrastructural analyses as well as functional assays were performed to determine whether or not the C-H lysosomal abnormality was expressed in the different lymphocyte subpopulations. B cells expressed the C-H defect following activation and differentiation. All of the Leu-7+ cells and a significant proportion of the Leu-3+ cells displayed the C-H abnormality. These Leu-3+ cells share the NK lineage characteristics of granular lymphocyte morphology and the capacity to bind to NK cell targets. In contrast, the C-H abnormality was not observed in non-NK target-binding cells with T helper phenotype, in which clusters of lysosomes formed a normal Gall body. Moreover, T cell functions were unimpaired in C-H patients. These observations raise the issue of the lineal relationship between granular and nongranular lymphocytes typed as T cells on the basis of cell surface antigen markers.

A LTHOUGH the genetic defect in the Chediak-Higashi (C-H) syndrome remains unexplained at the molecular level, several cellular abnormalities have been described in affected humans and animals; i.e., the beige mouse and aleutian mink. These alterations include changes in plasma membrane fluidity and in some cytoskeletal structures, but the presence of giant lysosomal granules within the cytoplasm is the most overt abnormality. The lysosomal abnormalities have been described in several nonhematopoietic cell types, including renal tubular cells, type II pneumocytes, chief cells and parietal cells of gastric glands, hepatocytes, neurons, melanocytes, and fibroblasts (reviewed). Lysosomal defects have also been detected in hematopoietic cells from several lineages, including granulocytes, monocytes, and megakaryocyte-platelets. In these bone marrow-derived cells, the lysosomal defect appears as a large cytoplasmic inclusion formed through fusion of multiple azurophilic granules. Heterophagocytosis and autophagocytosis contribute to the structural complexity and to the polymorphism of the C-H lysosomal body. The lysosomal abnormality is associated with impaired leukocyte function, which may explain some of the characteristics of the C-H syndrome and particularly the susceptibility to viral and bacterial infections.

The C-H abnormality does not appear to affect all of the blood lymphocyte subpopulations, but its expression has not been defined precisely. There is evidence suggesting that some T cell functions, such as mitogen responses and lectin-induced cytotoxicity, may be normal, whereas natural killer (NK) function and antibody-dependent cell-mediated cytotoxicity (ADCC) are impaired.Granular lymphocytes responsible for the latter functions have been shown to display the C-H lysosomal abnormality. Lysosomal abnormalities have been found in plasma cells from beige mice, but there is no information regarding the expression of the C-H defect in human B cells.

The present study was designed to determine the expression of the C-H lysosomal abnormality in human peripheral blood lymphocytes of various lineages by means of morphological, cytochemical, and functional analyses of purified subpopulations of T, B, and NK cells. B cells were identified by the HB-2 monoclonal antibody that reacts with a cell surface antigen expressed selectively by mature cells of B lineage. NK cells were identified through the expression of the Leu-7 (HNK-1) surface marker. The Leu-7 monoclonal antibody was selected from other available markers because this marker of NK cells is expressed on granular lymphocytes with and without cell surface antigens characteristically expressed by T cells. T cells were selected according to the expression of the Leu-3+ helper marker because markers of the NK lineage (ie, Leu-7, M1, Leu-11) are infrequently expressed on Leu-3+ cells, whereas Leu-2+ cells are often Leu-7+. When examined for intracellular distribution of lysosomes by means of cytochemical

From the Departments of Pathology and Pediatrics, and the Cellular Immunobiology Unit of the Tumor Institute, University of Alabama, Birmingham.

Supported by National Institutes of Health grants No. CA 16673, CA 25408, CA 13148, and CA 27197. A.V. is a recipient of the Rita C. and Solomon P. Kimerling Fellowship in Leukemia Research.

Submitted June 13, 1984; accepted Oct 8, 1984.
Address reprint requests to Dr Carlo E. Grossi, Department of Pathology, The University of Alabama in Birmingham, S234 Spain Wallace Bldg, Birmingham, AL 35294.
© 1985 by Grune & Strattion, Inc.

techniques, however, approximately 20% of the Leu-3' cells display scattered granules and are therefore similar to granular lymphocytes found in other T and non-T subsets. In the remaining Leu-3' cells, lysosomes are clustered in a single cytoplasmic area to form the so-called Gall body. Furthermore,granular Leu-3' cells form conjugates with NK cell targets (K562 or MOLT-4 cells), a property not shown by the nongranular Leu-3' cells. This characteristic has provided an additional marker for a more accurate analysis of the subset of cells with T helper phenotype in the C-H disease.

In this study we show that (1) the C-H lysosomal defect is expressed in B cells as a function of their activation; (2) the C-H lysosomal defect is found in all of the granular lymphocytes, independently of their surface phenotype; and (3) lymphocytes with T cell markers and lysosomal clusters forming the Gall body do not show the C-H abnormality.

MATERIALS AND METHODS

Subjects

The clinical history and results of the previous studies of the two patients with the C-H syndrome have been described elsewhere. Patient A is a 6'/2-year-old male child followed at the Children's Hospital and the University of Alabama, Birmingham. At the time of study he was free of overt infections and had received no therapy. Patient B is a 29-year-old male, seen in the Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md. Blood samples were obtained through the courtesy of Dr Anthony S. Fauci. At the time of study patient B was recovering from a urinary tract infection for which he had received antibiotic therapy and abdominal surgery.

Normal controls were studied in parallel with C-H patients. Blood samples were obtained from young adult volunteers. Informed consent was obtained from the healthy adults, patient B, and the parents of patient A.

Mononuclear Cell Suspensions

Mononuclear cells were separated from heparinized blood samples by Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) density gradient centrifugation and partially depleted of monocytes by adherence (one hour at 37 °C) to plastic surfaces.

Immunofluorescence

Monocyte-depleted mononuclear cells from C-H patients and from normal controls were stained with the monoclonal antibodies, Leu-3, Leu-7, or HB-2, which are specific for a subset of T cells comprising those with helper function, for granular lymphocytes with NK function, and for B cells, respectively. This step was followed by staining with fluorescein isothiocyanate (FITC)-conjugated goat antibodies specific for mouse immunoglobulins. Leu-3', Leu-7', or HB-2' lymphocyte populations were subsequently isolated by fluorescence-activated cell sorter (FACS IV, Becton Dickinson, Sunnyvale, Calif); forward light scattering was used to select for small lymphocytes. The degree of purity of the sorted populations was >98%, as determined by fluorescence microscopy.

The surface phenotype of NK cells was defined by monoclonal antibodies that recognize granular lymphocytes (Leu-7, Leu-11, VEP13, M1) and with the anti-pan T antibody, Leu-4. Coexpression of Leu-11, M1, or Leu-4 on Leu-7' cells was also analyzed by two-color immunofluorescence using rhodamine isothiocyanate (RITC)-labeled Leu-7 and FITC-labeled Leu-11 or Leu-4. Staining with unconjugated M1 was followed by FITC-conjugated goat antibodies to mouse γ2b.

Binding of Leu-3' Cells to NK Cell Targets

FACS-purified Leu-3' cells from the C-H patients and normal controls were mixed with K562 cells at a 1:1 ratio, in RPMI with 5% fetal calf serum. Cells were spun at 100 g for seven minutes and incubated as pellets at 37 °C to allow conjugate formation. The supernatant was subsequently discarded, and 0.5 mL of 1.25% glutaraldehyde in 0.1 mol/L cacodylate buffer, pH 7.4, was added to the pellet, which was gently resuspended in the fixative. After a 30-minute fixation at room temperature, cells were washed in cacodylate buffer, and cytocentrifuge preparations were made for cytochemical staining and for determination of the percentage of Leu-3' cells forming conjugates with K562 targets. Fixed preparations were also processed for ultrastructural analysis.

NK and Anomalous Killer (AK) Cell Functions

The NK function of Leu-7', Leu-7', and Leu-3' cells was tested against K562 cells in a chromium release assay, as described elsewhere. The AK function of Leu-7' cells was assayed. FACS-purified Leu-7' cells (10⁶) were cultured with the same number of mitomycin-treated Leu-7' cells from a normal allogeneic donor for five days to induce AK activity. This was tested at the end of the culture period in a chromium release assay against ¹¹⁵I-labeled K562 cells.

Phytohemagglutinin (PHA) Stimulation

Unfractionated mononuclear cells or FACS-purified Leu-3' cells (2 × 10⁶) were cultured in the presence of PHA-P (5 μg/mL) for three days. ¹H-thymidine uptake was determined as a measure of cell proliferation. Some cultured cells were fixed with glutaraldehyde for cytochemical staining.

Pokeweed Mitogen (PWM)-Driven B Cell Differentiation

FACS-purified HB-2' cells from C-H patients (2 × 10⁶) were cultured with allogeneic Leu-3' cells (2 × 10⁵) and monocytes (2 × 10⁵) from a normal donor for seven days in the presence of PWM (20 μg/mL). Harvested cells were spun onto glass slides, fixed with acetic ethanol, and stained with FITC-conjugated goat-anti-human Ig for 30 minutes at room temperature. The percentage of plasma cells was evaluated in these preparations. Other cultured cells were fixed with glutaraldehyde for cytochemical staining and for ultrastructural analysis.

Cytochemical Localization of Acid Hydrolases

All cell samples were fixed in suspension with 1.25% glutaraldehyde in 0.1 mol/L cacodylate buffer for 30 minutes at room temperature and rinsed twice in phosphate-buffered saline. Cytocentrifuge cell preparations were incubated for one hour at 37 °C in substrates for the cytochemical localization of acid phosphatase (AP), beta-glucuronidase (B-G), or alpha-naphthyl-acetate esterase (ANAE), as detailed elsewhere. Briefly, for AP staining, slides were incubated in a substrate consisting of naphthol-AS-B Phosphate buffer, pH 5.2. For B-G staining, the substrate was naphthol-AS-B glucuronide and hexazonized pararosanilin in acetate buffer, pH 5.2. ANAE activity was revealed by incubation in a substrate containing alpha-naphthyl acetate and exazonized pararosanilin in phosphate
RESULTS

HB-2' B Cells

Less than 10% of the HB-2' cells from normal controls were positive for acid hydrolases (Fig 1A). Large cells, accounting for <1% of the total B cells, stained diffusely for all of the acid hydrolases tested and presumably represented activated B cells or plasmablasts. Approximately 10% and 50% of the HB-2' cells from patient A and patient B, respectively, displayed a focal positive staining for all of the lysosomal enzyme activities (Fig 1B). This finding suggested that in comparison with normal subjects, a larger number of preactivated B cells were present in C-H patients, possibly as a consequence of frequent infections.

In order to test the possibility that expression of the C-H abnormality could be related to active lysosomal biogenesis during B-cell activation or maturation, FACS-purified HB-2' cells from patient B were cultured with T cells and monocytes from a normal allogeneic donor, in the presence of PWM. In this system, cells expressing lysosomal defects would be the

buffer, pH 5.0. (All of the above reagents were purchased from Sigma.)

Electron Microscopy (EM) and EM Cytochemistry

All cell suspensions processed for ultrastructural analysis were fixed with 1.25% glutaraldehyde in 0.1 mol/L cacodylate buffer, pH 7.4, and washed in the same buffer. After 1% osmium tetroxide postfixation, cell samples were stained "in toto" with 1% uranyl acetate, dehydrated through an ethanol series and embedded in Spurr resin (Ladd Research Industries, Burlington, Vt).24

For the ultrastructural localization of AP activity, fixed cells were incubated for one hour at 37 °C in a substrate consisting of cytidin monophosphate (Sigma) and lead nitrate in acetate buffer, pH 5.1.44 Following incubation, cells were washed twice in cacodylate buffer and processed as above for resin embedding. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Philips EM 201 electron microscope.

HB-2' B Cells

Less than 10% of the HB-2' cells from normal controls were positive for acid hydrolases (Fig 1A). Large cells, accounting for <1% of the total B cells, stained diffusely for all of the acid hydrolases tested and presumably represented activated B cells or plasmablasts. Approximately 10% and 50% of the HB-2' cells from patient A and patient B, respectively, displayed a focal positive staining for all of the lysosomal enzyme activities (Fig 1B). This finding suggested that in comparison with normal subjects, a larger number of preactivated B cells were present in C-H patients, possibly as a consequence of frequent infections.

In order to test the possibility that expression of the C-H abnormality could be related to active lysosomal biogenesis during B-cell activation or maturation, FACS-purified HB-2' cells from patient B were cultured with T cells and monocytes from a normal allogeneic donor, in the presence of PWM. In this system, cells expressing lysosomal defects would be the
patient’s activated B cells. After seven days in culture, cells were stained for the immunofluorescence localization of cytoplasmic immunoglobulin and for the cytochemical localization of acid hydrolases. Analysis by fluorescence microscopy revealed that approximately 2% of the cells were positive for cytoplasmic immunoglobulin, and similar percentages of cells displayed the C-H abnormality when stained for the cytochemical localization of acid hydrolases (Fig 2). At the EM level, a large lysosomal inclusion staining for AP was found in large, plasmablast-like cells.

**Leu-7** Granular Lymphocytes (NK Cells)

Leu-7 cells from normal controls stained for acid hydrolases with a pattern of positive granules either scattered throughout the cytoplasm or concentrated in the nuclear notch (Fig 3A). All Leu-7 cells from both C-H patients displayed a single, large cytoplasmic inclusion, strongly staining for lysosomal enzyme activities (Fig 3B). At the EM level, the C-H inclusion of Leu-7 cells appeared as a large membrane-bound granule containing a heavily electron-dense matrix (Fig 4a). Myelin figures and mitochondrial residues were occasionally found in the electron-translucent space commonly seen between the matrix and the limiting membrane (Fig 4b). AP activity was detected in this electron-translucent space between the limiting membrane and the electron-dense core of the C-H inclusion (Fig 4c), while the latter failed consistently to stain for AP.

A panel of NK-reactive monoclonal antibodies was used to determine the surface phenotype of NK cells from patient A. Two-color immunofluorescence was used to examine for coexpression of surface markers. As shown in Table 1, the percentages of cells positive for each of the markers and the surface phenotype characteristics were similar to normal controls.

Both C-H patients had previously been studied for NK activity, which was found to be profoundly depressed.

We repeated this functional assay in patient B, using FACS-purified Leu-7 or Leu-7 cells, and confirmed that his Leu-7 cells exhibited impaired NK function (Table 2).

**Leu-3** T Cells

When stained for the localization of acid hydrolase activity, approximately 20% of the Leu-3 cells from normal donors displayed a cytochemical pattern indistinguishable from that of granular lymphocytes from other subsets; the remaining Leu-3 cells were stained in a single, dot-like localization (Fig 5A). In contrast, all of the Leu-3 cells from both C-H patients displayed a dot-like pattern of cytochemical staining (Fig 5B). This suggested that the Leu-3 granular lymphocytes might express the C-H lysosomal abnormality. Ultrastructural analysis of AP localization revealed that, similar to normal controls, the majority of C-H Leu-3 cells displayed a cluster of discrete AP-positive lysosomes in a single cytoplasmic area (Fig 6). In addition, evidence for the C-H abnormality (lysosomal fusion) was detected in a small proportion of Leu-3 cells.

Normal Leu-3 cells, which formed conjugates with K562 cells, displayed a granular pattern of cytochemical staining (Fig 7A), whereas all of the C-H cells...
Table 1. Cell Surface Antigens Expressed by NK Cells in C-H Patient and Normal Controls

<table>
<thead>
<tr>
<th>Donor</th>
<th>Leu-7</th>
<th>Leu-11</th>
<th>VEP13</th>
<th>M1</th>
<th>Leu-4</th>
<th>Leu-11</th>
<th>M1</th>
<th>Leu-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-H patient A</td>
<td>14.5</td>
<td>16.3</td>
<td>17.8</td>
<td>37.6</td>
<td>58.3</td>
<td>68.4</td>
<td>62.5</td>
<td>46.2</td>
</tr>
<tr>
<td>Controls (n = 18)</td>
<td>11.4 ± 7.5*</td>
<td>11.3 ± 6.8</td>
<td>12.4 ± 5.4</td>
<td>ND</td>
<td>58.8 ± 19.2</td>
<td>58.6 ± 18.8</td>
<td>84.0 ± 3.0</td>
<td>41.3 ± 5.4</td>
</tr>
</tbody>
</table>

*Mean values are expressed as ± SD.

Table 2. NK Function of Lymphocyte Subpopulations in C-H Patient and Normal Control

<table>
<thead>
<tr>
<th>Donor</th>
<th>Cell Subsets*</th>
<th>1/20</th>
<th>1/10</th>
<th>1/5</th>
<th>1/2.5</th>
<th>1.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-H patient B</td>
<td>Leu-7'</td>
<td>5.1</td>
<td>3.5</td>
<td>3.0</td>
<td>0.1 ± 0.05</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Leu-7</td>
<td>3.2</td>
<td>0.6</td>
<td>0.7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Leu-3'</td>
<td>5.0</td>
<td>0.5</td>
<td>0.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>Leu-7'</td>
<td>67.4</td>
<td>50.8</td>
<td>30.2</td>
<td>13.4 ± 2.2</td>
<td>2.0 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>Leu-7</td>
<td>11.6</td>
<td>8.3</td>
<td>1.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Leu-3'</td>
<td>ND</td>
<td>0.5</td>
<td>0.3</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

*FACS-purified cell populations.  
†51Cr-labeled K562 cells were used as NK targets.  
‡Mean values ± SD from assays in triplicate.

binding to NK targets stained in a single focal localization (Fig 7B). Analysis of the C-H Leu-3' conjugate-forming cells at the ultrastructural level (Fig 8) revealed that the lysosomal abnormality was indistinguishable from that found in the Leu-7' granular lymphocytes (Fig 4).

Since morphological analysis suggested that the majority of C-H Leu-3' cells did not display lysosomal alterations, function of T cells was tested in order to detect possible abnormalities. Following PHA stimulation in the presence of allogeneic monocytes, C-H Leu-3' cells showed a significant level of proliferation (22,000 cpm vs 56,000 in a normal control). Furthermore, in contrast with the observations on C-H B cell blasts, cytochemical analysis of PHA blasts failed to show any evidence of lysosomal fusion.

Following mitogen or alloantigen stimulation, activated T cells may acquire the capability of lysing NK targets (anomalous killer [AK] function). To determine AK function, Leu-7' cells from C-H patient A and from a normal control were stimulated with mitomycin-treated Leu-7' cells from an allogeneic donor and subsequently tested for their cytotoxic activity against 51Cr-labeled K562 cells. Leu-7' (NK) cells were consistently below 1% in the Leu-7'–depleted populations both before and after culture. A comparable level of AK activity was found for C-H and control.
A

Fig 7. FACS-purified Leu-3⁺ cells were admixed with K562 cells and allowed to form conjugates (see Materials and Methods). (A) Leu-3⁺ conjugate-forming cells from a normal control display a scattered granular pattern of AP cytochemical staining. (B) Leu-3⁺ conjugate-forming cells from a C-H patient stain for ANAE in a single, focal, cytoplasmic localization (original magnification x900; current magnification x522).

Leu-7 cells (Table 3). This confirms previous observations on C-H patients showing that T cell functions were essentially normal.

We have reported elsewhere that K-562 conjugate-forming Leu-3⁺ cells do not kill the targets to which they bind. Accordingly, neither Leu-3⁺ cells from C-H patient B or from a normal control exhibited NK function in a chromium release assay (Table 2).

DISCUSSION

The present study has revealed the presence of the C-H lysosomal defect among B cells, NK cells, and lymphocyte subpopulations generally classified as T cells. Among the B cells from two C-H patients, 10% to 50% displayed a focal cytochemical reaction for acid hydrolases, whereas in normal controls <10% of the B cells stained for lysosomal enzymes. When C-H B cells were stimulated in the presence of PWM, normal allogeneic T cells, and monocytes, both activated B lymphoblasts and plasma cells were found to display prominent C-H inclusions. These observations suggest that the C-H lysosomal defect is maximally expressed following B cell activation and active lysosome formation.

NK cells were separated according to the expression of the Leu-7 antigen. This marker is found on granular lymphocyte subpopulations that may (1) coexpress T cell markers (Leu-4⁺, Leu-2⁺), (2) form E-rosettes but lack T cell antigens (Leu-5⁺, Leu-4⁻), or (3) lack T and B cell markers and hence fall within the “third population” cells. All of the Leu-7⁺ cells from normal controls displayed scattered azurophilic granules that contained acid hydrolases. Our cytochemical studies revealed that all of the Leu-7⁺ cells in C-H patients contained a single large cytoplasmic inclusion, strongly positive for all of the acid hydrolases tested. At the fine structural level, the C-H lysosomal body appeared as a giant membrane-bound granule containing a heavily electron-dense central matrix. An electron-translucent space between the limiting membrane and the central matrix contained residues of cytoplasmic organelles and AP activity. The structure of the cytoplasmic inclusion in C-H Leu-7⁺ cells suggests that, as in other cell types (granulocytes and monocytes), autophagocytosis, together with lysosomal fusion, contributes to the formation of the C-H body.

Analysis of surface phenotype and function confirmed that NK cells in C-H patients are normal in number and surface phenotype but have reduced cytotoxic function. This suggests that integrity of the lysosomal apparatus is important for cell-mediated cytotoxicity.

Leu-3⁺ cells were selected as a representative T cell population because, in contrast to cells of the Leu-2⁻ subset, they rarely express cell surface markers typical of NK cells. However, on morphological grounds, two populations of Leu-3⁺ cells can be distinguished according to the cytoplasmic distribution of lysosomal granules: (1) cells in which all lysosomes are clustered in a single cytoplasmic area to form the so-called Gall body (~80% of the Leu-3⁺ cells) and (2) cells with dispersed granules (~20% of the Leu-3⁺ cells). The latter are morphologically similar to the granular lymphocytes found in other T cell or non-T cell subsets and they share the capacity to bind to NK target cells, such as K562 cells.

Ultrastructural localization of AP activity showed that, in the majority of C-H Leu-3⁺ cells, no fusion...
occurred in the lysosomal clusters forming Gall bodies. Furthermore, no lysosomal fusion was shown by cytochemical staining of PHA-induced lymphoblasts from Leu-3' cells. However, in C-H patients, Leu-3' lymphocytes with multiple granules could not be detected by cytochemical staining for lysosomal enzymes, whereas such cells were found in normal controls. This suggested that the Leu-3' granular lymphocytes might express the C-H lysosomal abnormality. To examine this possibility, we took advantage of the ability of the C-H T cells that display Gall bodies, lack NK cell lineage characteristics, and appear to be morphologically and functionally normal in C-H patients. An alternative explanation for the lack of lysosomal fusion in Leu-3' cells marked by the Gall body could be that the nature of the granules forming this structure is different from that of the granules scattered in the cytoplasm of Leu-3' granular lymphocytes. However, the fact that both types of granules display structural and enzymatic features of primary lysosomes does not support this possibility.

In conclusion, the data indicate that the granular lymphocytes expressing T cell and NK cell lineage markers also display the C-H abnormality and suggest that this population of cells is developmentally divergent from the population of T cells that display Gall bodies, lack NK cell lineage characteristics, and appear to be morphologically and functionally normal in C-H patients.

ACKNOWLEDGMENT

We would like to thank Lilian Blutcher, Kathi Goodwin, and Gail Phillips for expert technical assistance. The expert secretarial work of Jane Hamner is gratefully acknowledged. We also thank Dr Anthony S. Fauci for providing blood samples from one patient and for critical reading of the manuscript.

REFERENCES

10. Davis WC, Spicer SS, Greene WB, Padgett GA: Ultrastructure of bone marrow granulocytes in normal mink and mink with the homolog of the Chediak-Higashi trait of humans. II. Cytoplasmic
23. Abo T, Roder JC, Abo W, Cooper MD, Balch CM: Natural killer (HNK-1) cells in Chediak-Higashi patients are present in normal numbers but are abnormal in function and morphology. J Clin Invest 70:193, 1982
27. Abo T, Landay A, Cooper MD, Balch CM: A B-cell differentiation antigen expressed on mature B cells but not on pre-B and plasma cells, identified by a monoclonal antibody (HB-2). (submitted for publication)
35. Velardi A, Grossi CE, Cooper MD: A large subpopulation of lymphocytes with T-helper phenotype (Leu3/T4-') exhibits the property of binding to NK cell targets and granular lymphocyte morphology. J Immunol 134:58, 1985
Expression of the Chediak-Higashi lysosomal abnormality in human peripheral blood lymphocyte subpopulations

CE Grossi, WM Crist, T Abo, A Velardi and MD Cooper