A diminished chemotactic response was observed with the neutrophils of a patient with the Chediak-Higashi syndrome, who was not in the accelerated phase of the disease. An abnormally low release of myeloperoxidase from these cells during phagocytosis was also noted; this resulted in a decreased iodination capacity and probably also caused the defect in the intracellular killing of bacteria by the neutrophils. The level of cyclic AMP in these cells was elevated, but decreased after treatment with ascorbate either in vitro or in vivo. During ascorbate therapy, the bactericidal activity of the neutrophils normalized, whereas the chemotactic response remained low. Nevertheless, the patient had significantly less infections during ascorbate therapy. The bleeding tendency, due to a storage-pool disorder of the Chediak-Higashi platelets, was unaffected by treatment with ascorbate. The patient's lymphocytes did not display any activity in antibody-dependent lymphocytotoxicity. This defect was not affected by treatment with ascorbate either.

The Chediak-Higashi (CH) syndrome is an autosomal recessive disorder, characterized by partial oculocutaneous albinism, recurrent pyogenic infections, increased bleeding tendency, and the occurrence of a "lymphoma-like accelerated phase." The presence of giant granules in many granule-containing cells is pathognomonic for the disease. The susceptibility to infections may be explained by the neutropenia and the poor response to chemotactic stimuli of the polymorphonuclear leukocytes (PMN) of these patients. Moreover, the delay in fusion of the PMN granules with the phagocytic vacuoles may be the cause of the decreased bactericidal capacity of these cells.

The bleeding tendency of CH patients is caused by disturbances in platelet aggregation. This dysfunction seems to be due to a storage-pool defect of the platelets. Most investigators found no abnormalities in the reactivity in vitro to mitogens, antigens, or allogeneic lymphocytes by lymphocytes from CH patients. The recently described impairment of natural killer-cell function in the beige mouse, an animal homologue of the CH syndrome, may be related to the tumor development in these animals.

The exact nature of the defects in CH PMN is unknown, but the abnormalities observed are believed to be related to disturbances in microtubule function. First, it is well known that chemotaxis as well as degranulation of PMN is regulated by the intracellular levels of cyclic nucleotides and that microtubules are also involved in these processes. Second, in PMN from CH patients, the content of cyclic AMP is abnormally high. Moreover, microtubule assembly, chemotactic responsiveness, and degranulation of these cells is abnormally low. And third, these disturbances have been (partially) corrected, both in vitro and in vivo, by treatment with cholinergic agonists or ascorbate. However, the efficacy of ascorbate therapy in CH patients has been disputed.

We have, therefore, investigated the effect of ascorbate on the metabolism and function of PMN, platelets, and lymphocytes from a CH patient. The functional abnormalities of the PMN of this patient were partially corrected by treatment with ascorbate in vitro, and almost totally in vivo. The platelet and lymphocyte abnormalities, however, were not restored by this treatment.

BLOOD DONORS

Case Report

The patient, born in January 1964 from healthy, nonrelated parents, is a mentally retarded boy, with a rotary nystagmus, photophobia, ocular albinism, and partial albinism of the skin. His hair is bright red with a silken gloss.
occurred frequently from the age of 5 yr. Several blood transfusions were needed. Splenomegaly was noted for the first time in December 1976.

No significant leukocytosis was observed in response to the various infections. The number of leukocytes was usually less than 3 \times 10^9/liter. The immunoglobulin levels were normal or slightly elevated. The results of routine laboratory tests for electrolytes, liver function, renal function and serum lipids were within normal limits. Serum lysozyme levels were elevated (2.5 \mu g/ml; normal values, 0.4–2.0 \mu g/ml).

The diagnosis of Chediak-Higashi syndrome was based on the clinical picture, the neutropenia, and the occurrence of giant granules in peripheral leukocytes and bone marrow cells. Since January 1977, the patient has been treated with ascorbic acid, 500 mg/day, orally.

Controls

Healthy adult blood donors were used as controls. Informed consent was obtained, and the study was conducted according to the Declaration of Helsinki.

MATERIALS AND METHODS

Zymosan (ICN Pharmaceuticals, Cleveland, Ohio) was opsonized by incubation in fresh serum, as described previously. The serum-treated zymosan was stored at –80°C.

Polymorphonuclear Leukocytes

Cell Isolation

Polymorphonuclear leukocytes were isolated from fresh, defibrinated blood by a previously described modification of the Blyum technique. Judged microscopically, the final cell suspensions contained 91% ± 5% (mean ± SD) neutrophils and 9% ± 5% lymphocytes. The yield was 80% of the PMN present in the defibrinated blood, and 95%–99% of the PMN were viable as judged by trypan-blue exclusion. The final suspension medium had a pH of 7.4 and contained 138 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 0.6 mM CaCl_2, 1.0 mM MgCl_2, 0.5% (w/v) human albumin, and 5.5 mM glucose.

Functional Studies

The uptake of ^14C-Staphylococcus aureus (strain Oxford) was measured by counting the radioactivity in the PMN after lysis of noningested bacteria with lysozyme.

Chemotaxis and random migration of purified granulocytes was measured in vitro, by a modification of the leading-front method of Zigmond and Hirsch. Intracellular killing of S. aureus was measured with a modification of the method described by Solberg.

The content and release of lysozyme and beta-glucuronidase was measured as described by Goldstein et al. The myeloperoxidase (MPO) activity was measured essentially as described by Klebanoff. The release of MPO was measured either as an increase in the cell-free supernatants or as a decrease of MPO in the cell pellets.

The release of the lysosomal enzymes was expressed as percentage of the total activity in PMN, measured in the presence of 0.2% (v/v) Triton X-100. Lactate dehydrogenase was measured as an indicator of cell viability; release of this enzyme was always less than 5%.

Metabolic Reactions

Oxygen consumption was measured with an oxygen electrode, as described previously. The formation of superoxide radicals was determined spectrophotometrically by the superoxide-dismutase-inhibitable reduction of cytochrome-c, as described before. Hydrogen peroxide production was measured according to Homann-Müller et al. Hexose monophosphate (HMP) shunt activity was measured by the liberation of ^14CO_2 from 1-^14C-glucose, with a modification of the method described by Pachman et al. Liodination of ingested zymosan particles was measured with a modification of the method described by Pincus and Klebanoff.

Cyclic AMP (cAMP) was measured as previously described. The assay was performed with a cAMP kit (The Radiochemical Centre, Amersham, U.K.). Each determination was performed in duplicate; the within-assay coefficient of variation was 9% and the between-assay coefficient of variation was 12%. The result of treatment with phosphodiesterase (Sigma Chemical Co., St. Louis, Mo.) proved that the material was >90% cAMP. Cyclic GMP (cGMP) was measured with a radioimmunoassay (cGMP assay kit, The Radiochemical Centre, Amersham, U.K.) based on the competition for binding to a specific antibody between cyclic GMP in the cells and tritium-labeled cyclic GMP, according to Kobayashi and Fang. The tritiated cGMP was further diluted 1:5 with Tris-EDTA (50 mM Tris-HCl, 4 mM EDTA, pH 7.5), and the antiseraum was diluted 1:4 with 2.5% bovine gammaglobulin (Sigma) to increase the detection limit of the assay. Each determination was performed in duplicate; the within-assay coefficient of variation was 19%.

Platelets

Cell Isolation

Platelet-rich plasma (PRP) was prepared by centrifugation of citrated blood (1 part of 3.8% trisodium citrate plus 9 parts of blood) for 15 min at 200 g and room temperature. Platelet-poor plasma (PPP) was prepared by centrifugation of PRP for 10 min at 2000 g and room temperature. Platelets were counted with a hemocytometer.

Functional Studies

Platelet aggregation was measured by a turbidometric method according to Born et al. in a Payton dual-channel module (Payton Associates, Buffalo, N.Y.) with PPP as blank. Aggregating agents included adenosine diphosphate (ADP) (Sigma), arachidonic acid (Sigma), ristocetin (Lundbeck and Co., Copenhagen, Denmark), and collagen suspension (from bovine achilles tendon).

Uptake of 5-hydroxytryptamine (serotonin) was measured as described by Hardeman et al. Platelets (200,000/cumm) were incubated at 37°C with 2.5 \mu M 5-hydroxytryptamine (Koch-Light, Colnbrook, U.K.) for 180 min. The decrease in 5-hydroxytryptamine concentration in the cell-free supernatant (8000 g, 1 min, 25°C) was measured, as well as the increase in the cell-bound concentration.

Metabolic Reactions

Intermediates of prostaglandin synthesis were measured by incubating PRP with 0.7 \mu M (1-^14C) arachidonic acid (specific activity 55.5 mCi/mmmole, The Radiochemical Centre, Amersham, U.K.) for 15 min at 37°C under continuous stirring. After acidification with 2 M citric acid to pH 3–4, lipids were extracted twice with an equal volume of ethyl acetate. The combined organic phases were evaporated, and the residue was dissolved in 100 \mu liter of chloroform:methanol (50:50, v/v) and used for thin-layer chromatography on silica-gel plates (Merck, Darmstadt, West Germany). Fractions were eluted with chloroform:methanol:acetic acid:water (90:6:1:0.7, v/v), and counted by liquid scintillation. For identification, purified prostaglandins (kindly provided by Dr. D.H. Nugteren,
Centrifugation over Ficoll-lsopaque (determined by rosette techniques with sheep red blood cells, the cells as targets). The percentage of peripheral T lymphocytes was measured by rosette techniques with anti-D-sensitized erythrocytes. Antibody-dependent lymphocytotoxicity sensitization in mixed lymphocyte cultures, T-cell-mediated cytotoxicity as judged by trypan-blue exclusion.

Preparation of Suspensions

The lymphocytes were prepared by treatment with carbonyl iron, as previously described. Monocytes (5%-25%) were removed by treatment with Na$_2$CrO$_4$-labeled P815 mastocytoma (ADL) was measured. The lymphocytes were isolated from defibrinated blood by centrifugation over Ficoll-lsopaque (d = 1.079 g/cu mm at room temperature). Monocytes (5%-25%) were removed by treatment with carbonyl iron, as previously described. The lymphocytes were suspended in bicarbonate-buffered (pH 7.4) RPMI 1640 medium (Gibco, Grand Island, N.Y.). The cell viability was more than 95%, as judged by trypan-blue exclusion.

Functional Studies In Vitro

The proliferative response to mitogens, antigens, and allogeneic lymphocytes was measured as described by du Bois. After sensitization in mixed lymphocyte cultures, T-cell-mediated cytotoxicity (CML) was measured with Na$_2$CrO$_4$-labeled lymphoblasts as target cells. Antibody-dependent lymphocytotoxicity (ADL) was measured with Na$_2$CrO$_4$-labeled P815 mastocytoma cells as targets. The percentage of peripheral T lymphocytes was determined by rosette techniques with sheep red blood cells, the percentage of Fc-receptor-bearing lymphocytes by rosette techniques with anti-D-sensitized erythrocytes.

RESULTS

Polymorphonuclear Leukocytes

Function and Oxidative Metabolism

The random migration of the CH PMN was within normal limits (Table 1). The chemotactic response of these cells, however, was diminished on three occasions. The uptake of $^{14}$C-labeled S. aureus bacteria was normal, but the intracellular killing of this strain of bacterium was decreased, notably during the first 30 min (see also Fig. 1).

Metabolic studies of resting CH PMN (Table 2) revealed an elevated oxygen consumption, but normal superoxide production and hydrogen peroxide formation. During phagocytosis of serum-treated zymosan, the oxygen consumption, hydrogen peroxide formation, and glucose-1-$^{14}$C-oxidation were within the normal ranges, and the formation of superoxide anions was increased.

<table>
<thead>
<tr>
<th>Table 1. Functional Studies of PMN</th>
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<tr>
<td>Normal PMN</td>
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<tr>
<td>Random migration*</td>
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<tr>
<td>Chemotaxis*</td>
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<tr>
<td>Phagocytosis†</td>
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<td>Intracellular killing‡</td>
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*In µm/70 min.
†In percent bacteria ingested per PMN per min (3 bacteria added per PMN).
‡In percent bacteria killed after 30 min (3-30 bacteria added per PMN).

The levels of the lysosomal enzymes beta-glucuronidase, lysozyme, and myeloperoxidase were normal in CH PMN (Fig. 2). During phagocytosis, the release of MPO, but not that of beta-glucuronidase, was strongly diminished (Fig. 2). As a result, the MPO-mediated iodination of zymosan particles by CH PMN was strongly decreased (Fig. 2 and Table 4). No differences in the iodination of resting normal or CH PMN was observed (n = 5; not shown). Iodination by monocytes was also decreased (not shown).

Cyclic Nucleotide Levels

Although there was a considerable variation in the cyclic AMP levels of the CH PMN, we confirmed the original observation of Boxer et al. that cyclic AMP levels in these cells are elevated (Tables 3 and 4). The level of cyclic GMP was normal (Table 4).

Effect of Ascorbate In Vitro on Neutrophil Function

Ascorbate in vitro had no significant influence on the level of cyclic AMP in normal PMN (Table 3). In contrast, the cyclic AMP level of CH PMN was decreased by ascorbate.

As shown in Table 3, ascorbate added in vitro significantly increased the chemotactic responsiveness of normal PMN. The effect of ascorbate on the chemotactic response of CH PMN could only be measured once; these cells were slightly stimulated, too (though not to a normal value).

Fig. 1. Intracellular killing of S. aureus by normal CH PMN before and during treatment in vivo with ascorbate. The number of viable bacteria in the PMN of 15 normal controls (mean ± SD) is given at various incubation times (shaded area), expressed in percentage of the initial number of viable intracellular bacteria (100% at t = 0). The intracellular killing by PMN from the CH patient before (O---O) and during (X---X) ascorbate therapy is also shown. PMN (4-5 x 10$^6$/ml) were incubated with S. aureus in a bacteria:granulocyte ratio of 3:30, in the presence of 10% (v/v) human AB serum.
Effect of Ascorbate Therapy on Neutrophil Function

The CH patient was treated for 3 yr with 500 mg of ascorbate per day orally. The concentration of ascorbate in the blood rose from 45 to 62 μmole/liter (these values are both in the normal range). At regular time intervals, chemotaxis, cyclic AMP levels, intracellular killing, MPO release, and iodination capacity of the PMN were tested.

During treatment with ascorbate in vivo, a stimulation of the mean chemotactic responsiveness of the CH PMN was noted ($p = 0.05$) (Table 4). On 5 occasions, cyclic AMP levels were normal during treatment, while cyclic GMP levels remained within normal limits (Table 4).

The release of myeloperoxidase was enhanced by ascorbate therapy from approximately 10% to a mean of approximately 50% of the release shown by normal PMN (Table 4). The functional activity of MPO, as measured by the iodination of zymosan particles,
increased during ascorbate therapy from about 25% to about 60% of control values (Table 4). This partial restoration of the MPO release and iodination capacity was accompanied by a complete normalization of the bactericidal activity (Fig. 1).

**Platelets**

**Platelet Function**

The bleeding time of the patient was prolonged (14 min; normals, <8 min). Platelet number, prothrombin time, activated partial thromboplastin time, thrombin time, and fibrinogen level were all normal. Electronmicrographs of CH platelets revealed a reduced number of dense bodies (not shown).

Addition of 1.1–3.2 μM ADP caused irreversible aggregation of normal platelets, but resulted in a decreased and reversible aggregation of the CH platelets on the two occasions tested. At 4.25 μM ADP, the CH platelets aggregated normally. Collagen (4 and 6 μg/ml) induced a diminished aggregation of the CH platelets as compared with normal platelets. With 0.23 mM and 0.58 mM arachidonic acid, the CH platelets failed to aggregate, in contrast with normal platelets. At 1.16 mM arachidonic acid, the CH platelets showed only a reversible aggregation, whereas normal

Table 3. Effect of Ascorbate on PMN in Vitro

<table>
<thead>
<tr>
<th></th>
<th>Normal PMN</th>
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<th>CH PMN</th>
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<tbody>
<tr>
<td></td>
<td>No Addition</td>
<td>With Ascorbate*</td>
<td>No Addition</td>
<td>With Ascorbate*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>cAMP†</td>
<td>2.7 ± 0.4 (n = 6)</td>
<td>2.9 ± 0.5 (n = 6)</td>
<td>Exp. 1</td>
<td>9.6</td>
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<tr>
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<td></td>
<td></td>
<td>5.6</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Exp. 2</td>
<td>38.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>19.0</td>
</tr>
<tr>
<td>Chemotaxis‡</td>
<td>70 ± 1 (n = 8)</td>
<td>85 ± 4 (n = 8)</td>
<td>42</td>
<td>52</td>
</tr>
</tbody>
</table>

* 10 mM ascorbate was added (pH 7.4).
† in pmole/10⁷ PMN.
‡ in μm/70 min.

Results are given as means ± SEM.

The effect of ascorbate on the chemotactic responsiveness of normal PMN was statistically significant (p < 0.001, t test for paired observations.)
platelets aggregated irreversibly. Ristocetin (1.5 mg/ml) induced normal aggregation of the CH platelets.

**Platelet Metabolism**

The serotonin content of the CH platelets was low (CH platelets 50 ng/10⁹ platelets; control 200–600 ng/10⁹ platelets). The serotonin uptake by the CH platelets, measured as the decrease of serotonin in the supernatant, was diminished, while the level of serotonin in the CH platelets decreased after 30 min of incubation with serotonin (Fig. 4). This latter finding indicates an increased intracellular breakdown of serotonin. These results are consistent with a storage-pool disorder of the CH platelets.

Determination of cyclic nucleotide levels in platelets revealed no abnormalities (not shown). The platelets of the patient showed a normal conversion of arachidonic acid into cyclooxygenase products, measured as thromboxane-B2 (patient, 9%; normal, 11% ± 2%, mean ± SD, n = 4) as well as into lipooxygenase products, measured as 12-hydroxy-eicosa-tetraenoic acid (HETE) (21% versus 28% ± 16%, mean ± SD, n = 4).

**Effect of Ascorbate**

Although incubation in vitro with 10 mM ascorbate slightly stimulated platelet aggregation induced by arachidonic acid, no effect of treatment with ascorbate in vivo was observed on the bleeding time or on the platelet aggregation.

**Immunologic Studies**

**In Vivo Parameters**

Delayed-type hypersensitivity skin tests with various antigens, i.e., PPD, candida, trichophyton, mumps, and varidase, were normal. Serum immunoglobulin levels (IgA, IgM, IgG) and complement levels (C1q, C2, C4, C3) were all normal.

**Lymphocyte Reactivity In Vitro**

After stimulation with various mitogens (phytohaemagglutinin, pokeweed mitogen, antilymphocyte serum, Concanavalin-A) and antigens (PPD, candida, trichophyton, varidase), the incorporation of ³H-thymidine was normal in the CH lymphocytes (not shown). The responder capacity, as well as the stimulator capacity, of CH lymphocytes in the mixed lymphocyte reaction was also normal (not shown). Normal amounts of T cells and Fe-receptor-bearing cells were found (not shown).

Cell-mediated lymphocytotoxicity, a function of T-killer cells, was also normal (the patient’s lymphocytes were tested towards the lymphocytes of two unrelated donors and caused 43%–60% ⁵¹Cr release, n = 2; normal donors, 49%–61%, n = 4). Antibody-dependent lymphocytotoxicity (ADL), mediated by (non-T,
non-B) lymphocytes, was estimated on 4 different occasions. (Fig. 5). The CH lymphocytes failed to show any ADL. In contrast, the monocyte-mediated cytotoxicity against anti-D-coated erythrocytes was normal (not shown).

**Lymphocyte Metabolism**

The CH lymphocytes had a normal level of cyclic GMP (674 fumol/10^7 cells, normal range, 400–700 fumol/10^7 lymphocytes) and a normal level of cyclic AMP (74 pmol/10^7 cells; normal range, 25–120 pmol/10^7 cells).

**Effect of Ascorbate**

Neither addition in vitro nor treatment in vivo with ascorbate had any effect on the observed defect in ADL.

**Clinical Effect of Ascorbate**

To evaluate the clinical effect of treatment in vivo with ascorbate, the number of days of fever were counted before and during therapy. Before treatment, 40 days of fever were registered in 1975, and 60 in 1976. During therapy, only 5 days were recorded in 1977, 8 in 1978, and 2 in 1979 (Fig. 6). The splenomegaly, present in the last 2 yr before treatment, disappeared in the first 3 mo of treatment.

No effect was observed, however, on the morphological abnormalities of the neutrophils, nor was any effect noted on the bleeding tendency. Until now, there has been no sign of an accelerated phase of the CH syndrome.

**DISCUSSION**

The observed defects in the chemotactic responsiveness and intracellular killing of *S. aureus* of the CH neutrophils are in accord with earlier reports. The high values of some parameters of the oxygen metabolism of CH PMN have also been described before. Normal amounts of myeloperoxidase, beta-glucuronidase, and lysozyme in CH PMN have been found by Stossel et al. and in the present study, but other investigators found decreased levels of some of these enzymes. Obviously, heterogeneity exists among CH patients.

General agreement exists on the defective release of the azurophilic enzyme MPO during phagocytosis. However, in the patient described here, the release of the azurophilic enzyme beta-glucuronidase was normal. This discrepancy might be explained by the finding that MPO is much more bound to the granule membrane than are the other granular enzymes. The decreased release of MPO may point to a membrane defect in CH PMN, rather than a defect in the enzyme-release mechanism. The recently described elevated membrane fluidity in CH PMN fits with this possibility. However, another explanation might be that beta-glucuronidase is not colocated with MPO in the azurophilic granules, but in a distinct population of lysosomes.

Although a selective defect in the uptake of zymosan particles was not excluded, the normal phagocyt...
rate for S. aureus suggests no defect in this respect. Furthermore, because the hydrogen peroxide production during phagocytosis and the content of MPO were normal, the defective release of MPO from the CH PMN most probably caused the strongly decreased iodination of zymosan particles by these cells. The latter finding differs from that reported in the literature. The oxygen metabolism and the release of lysosomal enzymes, apart from MPO, were not decreased in the patient described here; therefore, we regard the defective release of MPO into the phagosomes and the resulting abnormality in the MPO-mediated iodination reaction as the cause of the observed delay in microbial killing.

In contrast with Gallin et al., we confirmed the observations of Boxer et al. that cyclic AMP levels are elevated in CH PMN, and that ascorbate treatment, both in vitro and in vivo, normalizes cyclic AMP levels. In the presently described patient, however, neutrophil function was only partially restored. Nevertheless, a clear clinical effect was noted.

We confirmed, to a certain extent, earlier observations in which the platelet defect in CH patients is consistent with a storage-pool disease: both uptake and storage of serotonin were decreased. No abnormality in cyclic nucleotide levels was demonstrated in the platelets. A finding not hitherto reported is the observation of normal prostaglandin synthesis in CH platelets. Ascorbate treatment failed to correct the platelet abnormalities, as had previously been found by Boxer.

Several investigators have dealt with the immune response in CH patients, because malignancies, often observed in these patients, are also rather frequently encountered in immunodeficient patients. Most authors reported that cellular immunity and delayed-type hypersensitivity in CH patients are normal before the occurrence of the accelerated phase of the disease. In some instances, however, a diminished delayed-type skin reactivity and a moderately decreased phytohemagglutinin response were noted. In one patient, ADL appeared to be normal. However, in this report the authors did not define the target cells used in their ADL assay. Therefore, it cannot be decided which cells exhibited the ADL activity. In the patient described in the present report, all immunologic parameters were normal except for a consistent and profound defect in the ADL. Ascorbate treatment had no effect on this activity, either in vitro or in vivo.

Several studies indicate that the antibody-dependent lymphocytotoxicity against nucleated cells is mediated by lymphoid killer cells (K cells), whereas monocytes have no lytic capacity in this system. On the other hand, monocytes are capable of lysing anti-D-sensitized erythrocytes. The distinction between these two types of antibody-dependent cellular cytotoxicity is also reflected by the present findings, i.e., a decreased antibody-dependent cytotoxicity by lymphocytes, but a normal cytotoxicity by monocytes. Since ADL is dependent on cells with Fc receptors for immunoglobulin-G, the defective ADL by the patient's lymphocytes might be explained by a decreased number of Fc-receptor-bearing lymphocytes. However, the number of EA-rosette-forming cells in the monocyte-depleted lymphocyte fraction was normal. Therefore, we conclude that the patient's lymphoid K cells have an intrinsic defect at some stage of their lytic cycle (which involves binding and fusion of effector and target cells followed by the induction of lysis). Roder and Duwe recently reported that the beige mouse lacks natural killer cells and killer-cell function; this probably reflects a similar defect. The cytotoxicity mediated by T cells (CML) was normal in the presently described CH patient. This may indicate that the process by which T cells kill their targets is in some way different from that of K cells. Whether this difference is manifested at the binding stage or at the actual lysis stage cannot be decided at present. Similar to the defects observed in phagocytes and platelets, which all point to a disturbance in membrane properties and/or microtubule function, it is conceivable that these abnormalities might be important in the antibody-dependent lymphocytotoxicity, too. It is feasible that the disturbance in the antibody-dependent lymphocytotoxicity has some causal relationship with the development of the "lymphoma-like" phase in CH patients, although any conclusion in this respect has to await further evidence.

As in the platelets, no abnormality in cyclic nucleotide levels was found in the patient's lymphocytes. Therefore, it is questionable whether the increased cyclic AMP levels in neutrophils are primarily responsible for the observed defects of the PMN. Malawista et al. have suggested that elevation of cyclic AMP levels may result from microtubule disassembly and/or membrane abnormalities. Furthermore, Haak et al. reported an increased membrane fluidity in CH PMN. These abnormalities in fluidity were corrected in vitro by oxidative agents, such as ascorbate or an H2O2-generating system. Because normal cells were unaffected by this treatment, these results indicate

*After completion of this manuscript, an article appeared by Roder et al., describing a diminished ADL and natural killer-cell activity by CH lymphocytes. Subsequently, we also found a defective natural killer-cell activity in the lymphocytes of the patient described in the present report.
that the membrane properties of CH PMN are different from normal. An alteration in membrane properties could also explain the abnormal fusion of azurophilic and specific granules found in these cells as well as the specific defect in the release of MPO from the PMN of the patient here described. In this concept, it is conceivable that the observed defects in platelet and lymphocyte function are caused by similar membrane abnormalities. Nevertheless, whatever the exact nature of the defect in CH cells, it is clear from the studies of both Boxer and our group that some CH patients may benefit from ascorbate treatment.

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