Natural Killer (HNK-1$^+$) Cells in Chediak-Higashi Patients Are Present in Normal Numbers but Are Abnormal in Function and Morphology

TORU ABO, JOHN C. RODER, WATARU ABO, MAX D. COOPER, and
CHARLES M. BALCH, *Cellular Immunology Unit of the Tumor Institute,
Departments of Surgery, Pediatrics and Microbiology, University of Alabama
in Birmingham, Birmingham, Alabama 35294; Department of Microbiology
and Immunology, Queen's University, Kingston, Ontario, Canada K7L 3N6;
Department of Pediatrics, Sapporo Medical College, Sapporo 060, Japan*

ABSTRACT Children with the Chediak-Higashi (CH) syndrome are known to have abnormalities of natural killer (NK) cell function. We used the HNK-1 monoclonal antibody that reacts specifically with human NK and K cells to distinguish whether this abnormality was due either to a numerical deficiency of NK cells or a defect in their ability to function. In eight CH patients, a significant proportion of their blood mononuclear cells (10–19%) expressed the HNK-1 differentiation antigen. The level of NK cells in the five children with CH syndrome was higher than for age-matched normal controls (15.8% vs. 5.8%, $P < 0.001$). When HNK-1$^+$ cells were isolated with a fluorescence-activated cell sorter, the NK cells from CH patients were a homogeneous population of lymphocytes with a single large granule rather than the multiple small granules seen in NK cells from normal individuals. The purified HNK-1$^+$ cells from the CH patients had minimal NK or K cell function. The CH syndrome thus includes a functionally defective population of NK cells that retain the capability of expressing the HNK-1 differentiation antigen.

INTRODUCTION

Children with the Chediak-Higashi (CH)[1] syndrome, an autosomal recessive disorder, have an increased frequency of infections and decreased pigmentation (1). The characteristic giant granules in the cytoplasm of melanocytes and granulocytes is associated with the functional derangement of these specialized cells. A selective deficiency of natural killer (NK) cell function has been described in CH patients that may be related to their susceptibility to infections and their increased risk of malignancy (2–5). It has been suggested that NK cells may be present but functionally defective in affected individuals (2–4). Alternatively, it is possible that CH patients lack normal numbers of NK cells. These two possibilities were examined with a mono-

---

[1] *Abbreviations used in this paper*: ADCC, antibody-dependent cell-mediated cytotoxicity; CH, Chediak-Higashi syndrome; FACS, fluorescence-activated cell sorter; NK, natural killer.
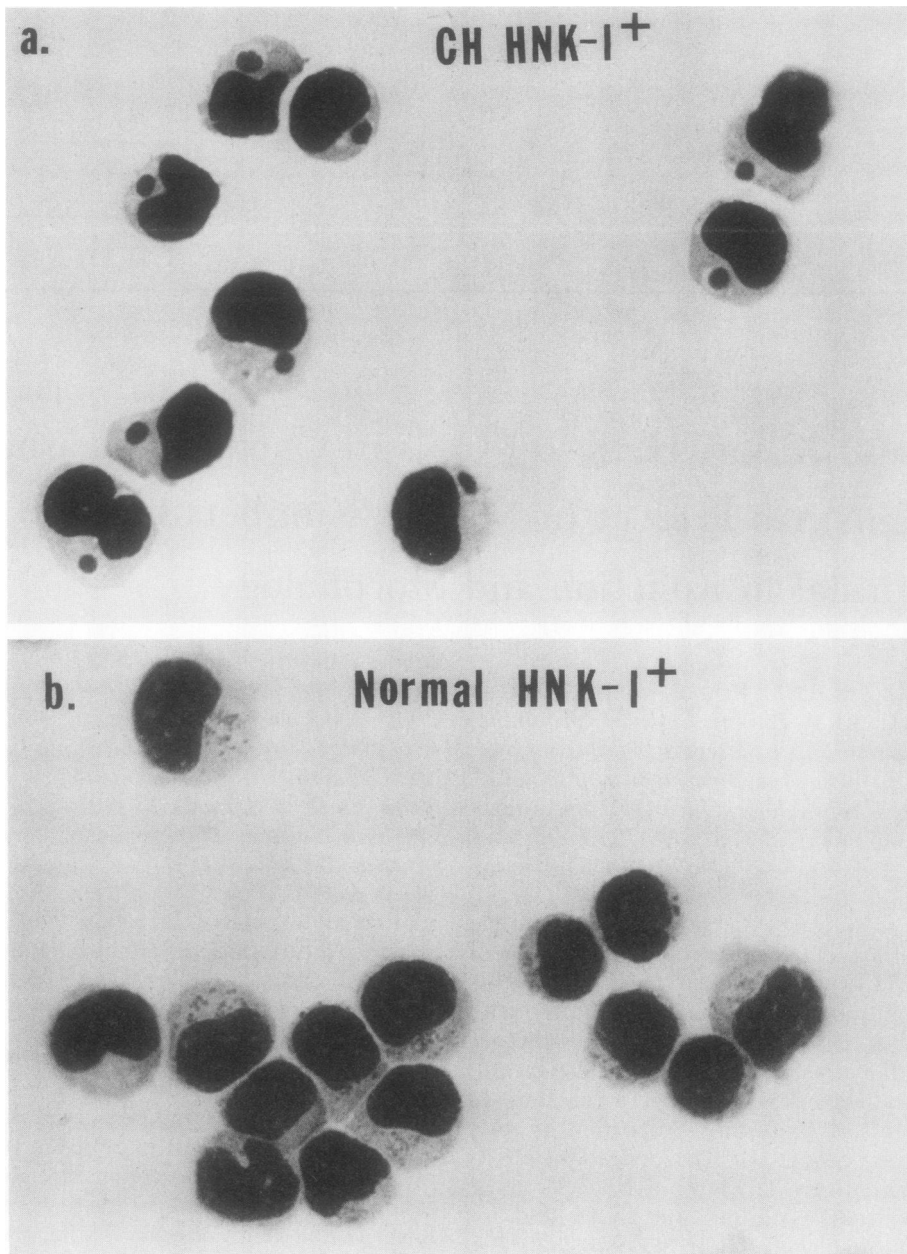
FIGURE 1 Characteristic large granule in NK cells from a CH patient. Mononuclear blood cells, stained by the HNK-1 antibody and fluoresceinated F(ab)$'_2$ anti-mouse immunoglobulin were separated into HNK-1$^+$ and HNK-1$^-$ cells by FACS. (a) HNK-1$^+$ cells from a 4-yr-old CH patient, N.S. (b) HNK-1$^+$ cells from a normal individual. (c) HNK-1$^-$ cells from the patient. A single large cytoplasmic granule was seen in virtually all HNK-1$^+$ cells from the patient, while the HNK-1$^+$ cells from the normal control contained multiple small granules. The HNK-1$^-$ cells from both patient and normal control were agranular small lymphocytes.

clonal antibody (HNK-1) that selectively reacts with a differentiation antigen on granular lymphocytes with NK and K cell function (6, 7).

METHODS

Patients. The CH patients and their clinical features have been described elsewhere (8, 9). The characterisitic giant

T. Abo, J. C. Roder, W. Abo, M. D. Cooper, and C. M. Balch
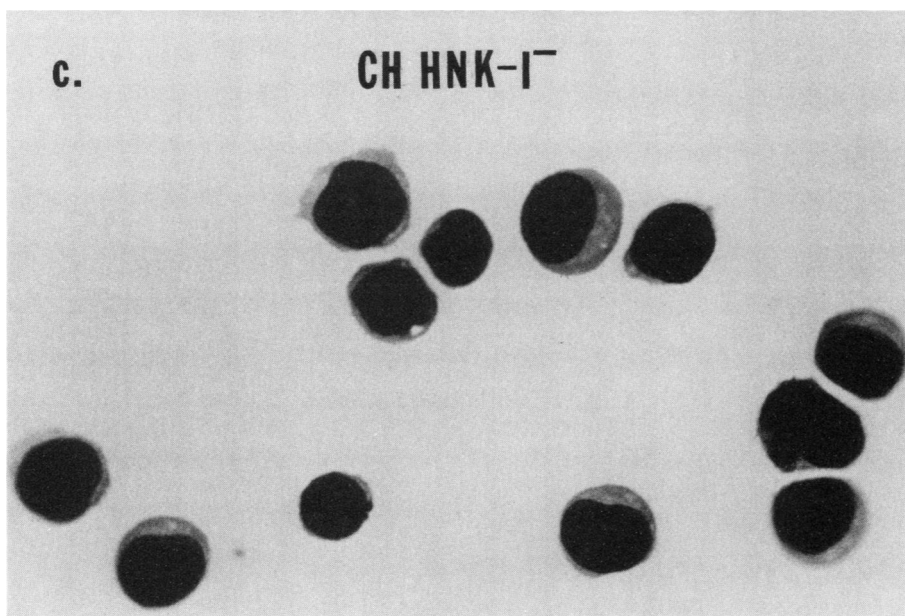
C. **CH HNK-1⁻**

FIGURE 1 (Continued)

granule formation of melanocytes and granulocytes was prominent in these patients, but the function of T and B lymphocytes appeared normal.

Cell preparation and immunofluorescence assay. Mononuclear cells were isolated from heparinized blood by Ficoll-Hypaque density gradient centrifugation. $HNK-1^+$ cells were enumerated by indirect immunofluorescence using the HNK-1 monoclonal IgM antibody (10 μg/ml) and fluoresceinated $F(ab)'_2$ anti-mouse immunoglobulin (0.4 mg/ml) as described previously (6, 7). Enumerations were performed using fresh cells from patients N.S. and R.Y., and on shipped blood cells removed on the previous day from the other CH patients. The frequency of $HNK-1^+$ cells was unchanged for control cell preparations over a 48-h period.

Functional assay. NK and K cell function was examined by a ^{51}Cr-specific release assay using K562 target cells and sensitized chicken erythrocytes, as described previously (6). Labeled target cells (10^4) were incubated at 37°C with effector cells suspended in a total volume 200 μl/well. Target to effector cell ratios (T:E) ranged from 1:10 to 1:2.5 in the NK cell assays and was a 1:10 ratio in all K cell assays. In the assay for K cell function, 10 μl of rabbit anti-chicken erythrocyte antibody was added to chicken erythrocytes to test for antibody-dependent cell-mediated cytotoxicity (ADCC) (6). After a 4-h incubation, 100 μl of supernatant was removed for counting. Percentages of ^{51}Cr-specific release = $2(S - R)/M - 2R$, where M is the maximum release of target cells, S is half of the top supernatant of experiments and R is half of top supernatant of target cell alone. Functional assays were performed using fresh blood cells from patient N.S., and with the same results using both fresh and shipped blood cells from patient H.N.

RESULTS

10–19% of mononuclear cells in the CH patients expressed the HNK-1 antigen (Table I). Since circulating NK cells (as defined by the HNK-1 antibody and functional assays) increase as a function of age (7), the values for CH patients were compared with age-matched controls. The proportion of $HNK-1^+$ cells in five young children with CH syndrome was significantly higher than in age-matched controls (15.8 vs. 5.8%, $P < 0.001$). In contrast, three older CH patients (ages 14–30 yr) had only slightly higher levels of HNK-

TABLE I
HNK-1⁺ Blood Mononuclear Cells Are Abundant in Patients with Chediak-Higashi Syndrome

Donor	Diagnosis	Age	Sex	% HNK-1⁺ cells
A. Children				
N.S.	CH	4	M	18
H.N.	CH	6	F	17
E.J.	CH	6	F	18
J.C.	CH	6	M	16
R.Y.	CH	7	F	10
Controls ($n = 8$)	Normal	4–7	M + F	5.8±2.3
B Adolescents and adults				
W.P.	CH	14	M	19
Le.R.	CH	29	M	19
La.R.	CH	30	M	15
Controls ($n = 23$)	Normal	15–30	M + F	14.3±5.3

A. NK Cell Assay (K-562)

HNK-1⁺ | HNK-1⁻ | Whole MNC

% ⁵¹Cr Specific Release

T:E Ratios

B. K Cell Assay (CRBC)

HNK-1⁺ | HNK-1⁻ | Whole MNC
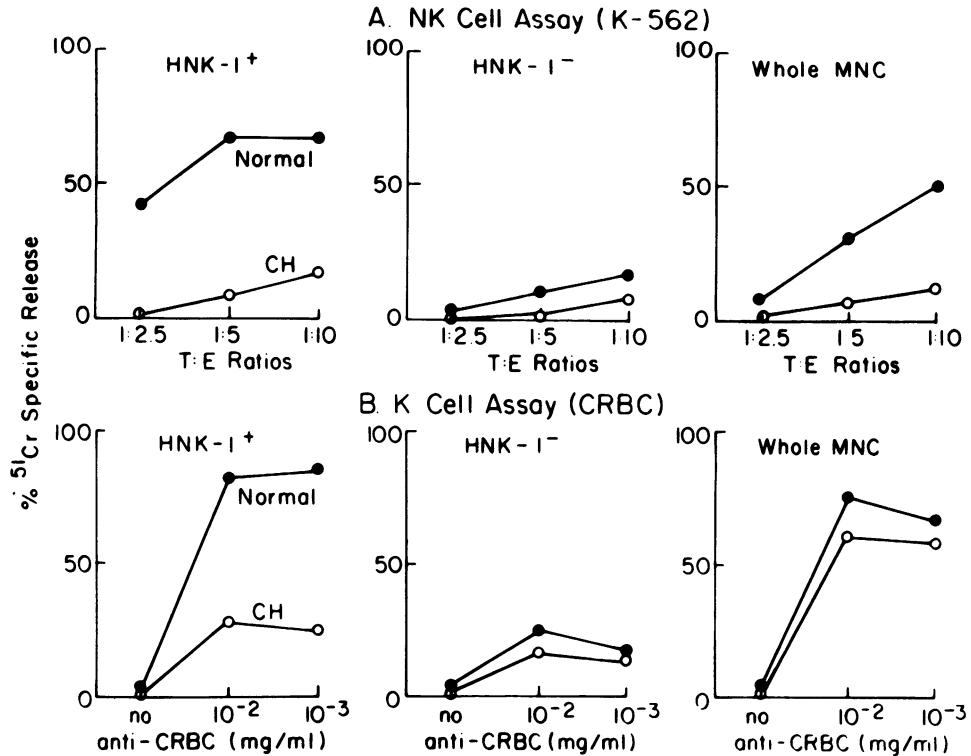
anti-CRBC (mg/ml)

FIGURE 2 Impairment of NK and K cell function by HNK-1⁺ cells from a CH patient. HNK-1⁺ cells from CH patient N.S. (- O -) were markedly defective in both NK and K cell functions compared with HNK-1⁺ cells from a healthy donor (- ● -). The sorted HNK-1⁺ and HNK-1⁻ cell fractions contained <1% monocytes; the whole mononuclear cell fraction in the patient contained ~15% monocytes.

1⁺ cells than did age-matched controls (17.7 vs. 14.3%, $P > 0.2$).

The morphological and functional characteristics of HNK-1⁺ cells were examined in greater detail using mononuclear blood cells from the 4-yr-old patient N.S. HNK-1⁺ and HNK-1⁻ cells were purified with a fluorescence-activated cell sorter (FACS) and then stained with May-Grünwald-Giemsa (Fig. 1). The sorted HNK-1⁺ cells were a homogenous population of lymphocytes containing a single giant granule (Fig. 1a). In contrast, HNK-1⁺ cells from normal individuals contained multiple small granules in their cytoplasm (Fig. 1b). HNK-1⁻ cells from the patient and from a normal individual were primarily small lymphocytes containing no granules (Fig. 1c). The morphological homogeneity of each sorted fraction exceeded 96%.

Studies of HNK-1⁺ cells from normal individuals have revealed the expression of HNK-1 antigen on both the cell surface and in the cytoplasm (7). The cytoplasmic HNK-1 staining appeared to increase as a function of NK cell maturation. We therefore examined the cytoplasmic distribution of HNK-1 deter-

minants in surface HNK-1⁺ cells from CH patients. A normal diffuse pattern of HNK-1 antigen expression was noted in the majority (60%) of cells from affected individuals. There was no concentration of HNK-1 antigen within the giant cytoplasmic granule.

Sorted HNK-1⁺ and HNK-1⁻ blood mononuclear cells were then examined for their NK and K cell functional activities using K562 target cells and sensitized chicken erythrocytes. The cytotoxic capability of effector cells was minimal in the 4-yr-old patient N.S. (who had 16% HNK-1⁺ cells) compared with a normal 19-yr-old male with 10% HNK-1⁺ cells (Fig. 2). The NK functional activity was depressed in the CH patient using both a total mononuclear cell preparation and the purified fraction of HNK-1⁺ cells. Interestingly, significant ADCC activity was observed in the whole mononuclear cell fraction (Fig. 2). This was probably due to a 15% contamination by monocytes, since minimal K cell activity was noted both in HNK-1⁺ and HNK-1⁻ cell fractions, which were depleted of large unstained cells during the cell sorter separations. This data is consistent with previous studies dem-

onstrating that ADCC activity by monocytes was intact in the CH patients, whereas K cell function in the lymphocyte fraction was abnormally low (4).

The dissociation between the HNK-1$^+$ cell level and absent NK cell function was also demonstrated in another CH patient H.N., using a discontinuous Percoll density gradient technique as previously described (10). HNK-1$^+$ cells from this 6-yr-old CH patient were enriched into a low density fraction similar to those of the normal donor. However, this fraction had a depressed NK cell functional capability compared with the control (data not shown).

DISCUSSION

The present results provide direct evidence in support of previous proposals (2–4) that the abnormality in CH patients is due to functionally defective NK cells. We have found that lymphocytes expressing the HNK-1 differentiation antigen and characteristic granular morphology, are present in normal or elevated levels in CH patients. The expanded population of circulating HNK-1$^+$ cells that we observed in young CH patients could reflect accelerated development of HNK-1$^+$ cells or excessive accumulation of functionally abnormal NK cells.

Highly enriched (96%), HNK-1$^+$ CH cells isolated on the FACS exhibited defective NK and ADCC function and were characterized by the presence of a single large granule in their cytoplasm. On the other hand neither macrophages nor T cells from CH patients exhibited giant granules (Fig. 1c and unpublished observations), whereas both effector cell types exhibited normal frequencies and cytolytic functions (Fig. 2 [4]). It seems likely, therefore, that giant granule in NK cells may be closely linked to the functional defect. The underlying mechanism of giant granule formation is not known but may involve an abnormal interaction between the lysosome membrane and microtubules (11).

The function of the HNK-1 antigen is not yet known but it is probably not involved directly in the NK-target cell interaction because NK activity was not inhibited by the HNK-1 antibody and inhibition of NK cytolysis by mild pronase treatment did not affect the expression of HNK-1 (6). It is interesting to note that the distribution of intracellular HNK-1 antigen in CH-NK cells was normal.

This study thus demonstrates that the recessive genetic defect underlying the CH syndrome affects lymphocytes expressing the HNK-1 differentiation antigen. Previous studies have revealed abnormalities of other granule-containing cells, e.g., granulocytes and melanocytes (1). The CH defect also affects functions of these other cell types rather than their population size. By contrast, T lymphocytes and macrophages in CH patients do not contain the giant granule, lack the HNK-1 antigen and exhibit normal function. Our studies identify the mononuclear blood cells displaying the characteristic giant granules, all of which express HNK-1 antigen, as functionally defective NK cells. These observations are consistent with the idea that NK cells belong to a separate pathway of lymphoid differentiation.

REFERENCES

1. Windhorst, D. B., and G. J. Padgett. 1973. The Chediak-Higashi syndrome and the homologous trait in animals. *Invest. Dermatol.* 60: 529–537.
2. Roder, J. C., T. Haliotis, M. Klein, S. Korec, J. R. Jett, J. Ortaldo, R. B. Herberman, P. Katz, and A. S. Fauci. 1980. A new immunodeficiency disorder in humans involving NK cells. *Nature (Lond.).* 284: 553–555.
3. Haliotis, T., J. Roder, M. Klein, J. Ortaldo, A. S. Fauci, and R. B. Herberman. 1980. Chediak-Higashi gene in humans I. Impairment of natural-killer function. *J. Exp. Med.* 151: 1039–1048.
4. Klein, M., J. Roder, T. Haliotis, S. Korec, J. Jett, R. B. Herberman, P. Katz, and A. S. Fauci. 1980. Chediak-Higashi gene in humans II. The selectivity of the defect in natural-killer and antibody-dependent cell-mediated cytotoxicity function. *J. Exp. Med.* 151: 1049–1058.
5. Dent, P. B., L. A. Fish, J. G. White, and R. A. Good. 1966. Chediak-Higashi syndrome. Observation on the nature of the associated malignancy. *Lab. Invest.* 15: 1634–1642.
6. Abo, T., and C. M. Balch. 1981. A differentiation antigen of human NK and K cells identified by a monoclonal antibody (HNK-1). *J. Immunol.* 127: 1024–1029.
7. Abo, T., M. D. Cooper, and C. M. Balch. 1982. Postnatal expansion of the natural killer and killer cell population in humans identified by the monoclonal HNK-1 antibody. *J. Exp. Med.* 155: 321–326.
8. Prchal, J. T., W. M. Crist, A. Malluh, A. Vitek, W. N. Tauxe, and A. J. Carroll. 1980. A new glucose-6-phosphate dehydrogenase deficient variant in a patient with Chediak-Higashi syndrome. *Blood.* 56: 476–480.
9. Roder, J. C., L. Laing, T. Haliotis, and D. Kozbor. 1981. Genetic control of human NK function. *In* NK cells: Fundamental Aspects and Role in Cancer. R. B. Herberman, editor. Elsevier/North-Holland Publishers, Amsterdam. In press.
10. Timonen, T., and E. Saksela. 1980. Isolation of human NK cells by density gradient centrifugation. *J. Immunol. Methods.* 36: 285–291.
11. White, J. G., and C. C. Clawson. 1979. The Chediak-Higashi syndrome: Microtubules in monocytes and lymphocytes. *Am. J. Hematol.* 1: 349–356.