Impaired Culture Generated Cytotoxicity with Preservation of Spontaneous Natural Killer-Cell Activity in Cartilage-Hair Hypoplasia

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ABSTRACT Recent studies of cartilage-hair hypoplasia (CHH), a form of short-limbed dwarfism, have shown that all affected individuals have a cellular proliferation defect that results in a cellular immunodeficiency. However, only a minority of CHH individuals suffer from severe, life-threatening infections. For this reason, relevant immune defense mechanisms that may be responsible for maintaining intact host defenses in the majority of CHH individuals were studied. Spontaneous and allogeneic culture-induced (mixed lymphocyte response-MLR) specific and nonspecific (NK-like) cytotoxic mechanisms were analyzed and correlated with lymphocyte subpopulations present in CHH and normal individuals. Spontaneous natural-killer (NK) activity was present at or above normal levels, but culture-induced specific cytotoxicity and NK-like cytotoxicity as well as NK-like activity by T cell lines were significantly reduced in CHH individuals. The generation of radiation-resistant cytotoxicity, which normally occurs during allogeneic MLR, was markedly diminished in CHH, and was correlated with the decreased proliferation observed in CHH cultures. Preservation of spontaneous NK activity and loss of all forms of culture-induced cytotoxicity was associated with an increase in the proportion of lymphocytes bearing a thymic independent NK phenotype ($OKM1^+$ $OKT3^-$ $Fc\gamma^+$ low-affinity E^+), and a significant decrease in thymic derived $OKT3^+$ cytolytic T cell subpopulations in CHH individuals. Therefore, an intact cellular cytotoxic effector mechanism has been identified in CHH (i.e., NK activity). Natural cytotoxicity may be of importance in maintaining host resistance to viral infections despite diminished thymic-derived effector mechanisms in cartilage-hair hypoplasia.

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INTRODUCTION

Cartilage-hair hypoplasia (CHH)[1] is an autosomal recessive form of short-limbed dwarfism, which is found in increased frequency in the Old Order Amish of the United States, as well as in the Finnish population (1, 2). It is characterized by short stature, sparse unpigmented hair, and cellular immunodeficiency (1–4). CHH individuals have decreased numbers of circulating B and T lymphocytes, negative delayed-type hypersensitivity reactions, and severely depressed proliferative responses to B and T cell specific antigens and mitogens. Impaired interleukin 2 (IL2) production and utilization have been found in studies on continuous T cell lines (CTCL), suggesting that the presence of a G_1 phase defect in the activation of CHH T cells (5).

The Amish cartilage-hair hypoplasia subjects investigated in our previous studies were healthy and infection free, in spite of their significant in vivo and in vitro immunologic abnormalities. Only a minority of CHH individuals suffer severe, sometimes fatal, viral infections (1–3). Thus, we sought to identify immunological mechanisms present in CHH that might account for this apparent paradox. Cytotoxicity against virally infected altered-self has been demonstrated by $OKT3^+$ $OKT8^+$ HLA-restricted T cytotoxic cells as well as by $Fc\gamma^+$ natural killer (NK) cells (6–9). Our previous studies noted decreased numbers of $OKT3^+$ (pan-T), $OKT4^+$ (helper/inducer), and $OKT8^+$ (suppressor/cytotoxic) T lymphocytes, and a large increase in the proportion of $OKM1^+$ lymphocytes in CHH in-

[1] *Abbreviations used in this paper:* CHH, cartilage-hair hypoplasia; CML, cell-mediated lympholysis; CTCL, continuous T cell lines; E_{AET}, sheep erythrocytes pretreated with 2-amino-ethylisothiouronium; $Fc\gamma^+$, cells bearing receptors for the Fc portion of IgG; IL2, interleukin 2; MLR, allogeneic mixed lymphocyte reaction; NK, natural killer; PBMC, peripheral blood mononuclear cells.

dividuals (4). Since many OKM1$^+$ lymphocytes mediate spontaneous NK activity (10–12), and OKT3$^+$ T cells are responsible for proliferation and specific allogeneic cell-mediated lympholysis (CML) in the MLR (12–14), we have evaluated and compared spontaneous NK activity with allogeneic culture-induced specific CML and nonspecific (NK-like) cytotoxicity in CHH and normal individuals. We found that spontaneous NK activity was preserved in CHH. However, allogeneic culture induced specific and NK-like cytotoxicity were markedly diminished in CHH. In addition, the induction of radiation-resistant cytotoxicity in the MLR did not occur in lymphocyte cultures from CHH patients. Since most CHH individuals are healthy and survive well into adulthood, these findings suggest a clinically significant role for spontaneous NK cytotoxicity in host defense.

METHODS

Patients. The nine Amish CHH individuals (mean age 17.4 yr, range 13–26) who participated in these studies were originally diagnosed by field teams from the Johns Hopkins Hospital, under the direction of Dr. V. A. McKusick, using physical examinations, family histories, pedigree analyses, and, in some cases, radiographic and hair sample analyses (1, 3). All individuals were clinically healthy at the time of study. Six affected subjects had a history of severe, prolonged varicella infection, and four of these individuals also had histories of severe repeated chronic respiratory tract infections. None of the patients studied received vaccinia or polio immunizations in childhood. Eight patients had blond body hair, and in three it was exceptionally sparse (lanugo). Age- and sex-matched unrelated normal individuals, as well as four unaffected siblings, served as controls. Blood samples were drawn in accordance with the guidelines established by the Institutional Review Board of University Hospitals of Cleveland.

Cell separation. Peripheral blood mononuclear cells (PBMC) were obtained from heparinized whole blood by Ficoll-Hypaque density gradient centrifugation. PBMC were washed three times, counted, and used for the NK assay and the mixed lymphocyte culture induced cytotoxicity studies. For surface marker analysis, macrophages were removed from PBMC by adherence to plastic in 10% fetal calf serum (FCS) in RPMI 1640 and 5% CO_2 for 30 min. Nonadherent cells, designated as peripheral blood lymphocytes (PBL), were decanted and used immediately for cell surface antigen studies. For surface marker analysis of purified T cells, PBMC were rosetted with 2-amino-ethylisothiouronium-treated sheep erythrocytes (E_{AET}), and E_{AET}^+ cells were isolated on Ficoll-Hypaque gradients, as previously described (4). Cell recovery after separation procedures was similar for both CHH and normal individuals.

Fluorescence staining. Subpopulation analysis was performed using monoclonal antibodies against cell surface determinants, as previously described (4). Briefly, OKT3 (pan-T), OKT4 (helper/inducer), and OKT8 (suppressor/cytotoxic) were purchased from Ortho Pharmaceutical, Raritan, NJ. OKT5 (suppressor/cytotoxic), OKIa1 (anti-Ia), OKM1, and directly fluorescein-labeled OKT3, OKT4, OKT8, OKM1 were generously provided by Dr. Patrick Kung (Ortho Pharmaceutical).

Incubation of PBL and E_{AET}^+ cells in 10% FCS with latex particles (0.81 μm, Difco Laboratories, Detroit, MI) for 30 min at 37°C was performed before staining to identify residual macrophages. For staining, 3–5 × 10^5 PBL or E_{AET}^+ cells were incubated in duplicate with unlabeled monoclonal antibody for 30 min at 4°C, washed three times in 5% bovine serum albumin, then incubated with rhodamine-conjugated goat anti-mouse IgG (Cappel Laboratories, Inc., Cochranville, PA) for 30 min at 4°C. Nonspecific fluorescence was assessed by use of a mouse monoclonal IgG of irrelevant specificity, and was usually <3%.

Sheep erythrocyte (E), E_{AET}, and Fcγ rosettes were determined on duplicate samples. For E and E_{AET} rosettes, 3 μl of 5% (vol/vol) untreated or AET-treated sheep erythrocytes, respectively, were added to 5 × 10^4 lymphocytes in 25% FCS. Cells were centrifuged at 100 *g*, incubated at 4°C for 30 min, and viable cells (>97%) were read under epifluorescence after the addition of acridine orange-ethidium bromide (4). Fcγ rosettes were determined after overnight incubation of 5 × 10^4 lymphocytes in serum-free media with 5 μl of 5% IgG-coated ox erythrocytes, as previously described (5).

Culture of CML effectors. One-way MLR were initiated by culturing PBMC in round-bottomed tubes in 3-ml vol at a cell concentration of 3 × 10^6/ml with either an equal number of irradiated (3,000 rad) autologous or allogeneic PBMC in RPMI 1640 containing 20% pooled AB serum. After 5 d, proliferation was assessed in 96 well microcultures by tritiated thymidine incorporation ([^{3}H]TdR; 0.5 μCi/well, 25 Ci/mmol, New England Nuclear, Boston, MA), as described previously (15). After 6 d, effector cells were washed, counted, and diluted for the cytotoxic assays.

Cytotoxicity targets. Autologous and allogeneic PBMC targets were maintained in culture for a total of 6 d in 20% AB serum in the absence of mitogens until they were required for the cytotoxic assays. The erythroleukemia cell line K562 was maintained in exponential growth culture.

Cytotoxic assays. Fresh or cultured effector PBMC were placed in round-bottomed wells of a microtiter tray with 5 × 10^3 ^{51}chromium-labeled (>450 mCi/mg, 1 mCi/ml, New England Nuclear) target cells at effector to target cell (E/T) ratios of 30:1, 15:1, and 7.5:1 in 150 μl RPMI 1640 and 20% FCS. The targets used were autologous or allogeneic PBMC, or K562. The tray was spun at 50 *g* for 5 min, incubated for 4 h at 37°C in 5% CO_2 in air, and then centrifuged at 150 *g* for 10 min. Aliquots (50 μl) of the supernatant were then removed and placed in glass tubes for counting in a gamma counter. Cytotoxic activity was quantitated as percentage of lysis:

$$\% \text{ lysis} = \frac{\text{cpm (experimental release)} - \text{cpm (spontaneous release)}}{\text{cpm (total release)} - \text{cpm (spontaneous release)}} \times 100.$$

Spontaneous release of ^{51}chromium from target cells was determined in the presence of medium alone, whereas total release was determined by lysis in 1% sodium dodecyl sulfate. Spontaneous release from targets was always <10%. Activity was linear over the range of effector-to-target ratios used. Cytotoxicity for autologous or allogeneic targets in autologous MLR-CML cultures was generally less than 5% of the cytotoxicity found in primed allogeneic MLR cultures directed against allogeneic targets. Autologous cytotoxicity in primed allogeneic cultures was less than 10% of the allogeneic cytotoxicity.

Radiation-resistant killing. Aliquots of fresh or cultured PBMC were irradiated (3,000 rad with a cobalt source) to

assess radiation-resistant killing, as previously described (15). Irradiated cells were treated identically to nonirradiated cells, except that radiation resistance experiments were performed using E/T ratios of 30:1 only. Intraassay coefficients of variation among triplicate samples were <3% for unirradiated and irradiated samples.

CTCL. Cell lines were initiated as previously described (5). Briefly, T cell blasts derived from phytohemaglutinin stimulated PBMC were maintained in continuous proliferation by feeding at 5-d intervals with 40% (vol/vol) interleukin-2-containing medium. IL2 was prepared from an irradiated, pooled MLR from 10 allogeneic individuals cultured in the presence of 3.75 μg/ml PHA-M (Difco Laboratories). After 48 h, the supernatant was harvested, filtered through a 0.45-μm filter, and stored at −20°C until used. Supernatants were tested for IL2 potency in a CTCL microassay, as previously described (5). After 20 d of culture, CTCL from two CHH and two normal individuals were tested for cytotoxicity against K562 targets as described above.

Statistical analysis. A two-tailed unpaired Student's *t* test was used to evaluate significance levels. Critical values of the sample correlation coefficient, *r*, were determined in a two-tailed test.

RESULTS

Cytotoxic mechanisms in CHH and normal individuals. Spontaneous NK activity was at or above normal levels in all CHH individuals studied (Fig. 1A). Fresh PBMC from eight CHH individuals lysed 44.3±5.0% (mean±SE) of K562 target cells in the 4-h NK assay at a 30:1 E/T ratio, compared with 36.4±3.9% lysis by fresh PBMC from eight normal individuals (Fig. 1A). The killing was linear for all E/T ratios tested, and no significant differences were found between the two groups.

In contrast, minimal MLR-induced specific cell mediated lympholysis could be generated in CHH individuals (4.6±2.1% at E/T 30:1), whereas PBMC from normal subjects sensitized in an MLR lysed 26.3±2.4% of the targets at the same E/T ratio ($P < 0.001$, Fig. 1A). Three CHH individuals had no detectable CML. Decreased MLR-induced proliferation was found against each of the allogeneic individuals used as specific targets in the CML assay (CHH = 1,548±335 cpm, normal controls = 13,007±4,629 cpm; $P < 0.005$; Fig. 1B). This was not due to a lack of HLA-D locus differences, since in this series, as in previous studies (3, 4), the proliferative response to a pool of 10 allogeneic individuals was significantly diminished (CHH = 3,531±609 cpm; normal = 24,262±2858 cpm; $P < 0.001$).

NK-like cytotoxicity against K562 was also assayed in the 6-d allogeneic MLR cultures. NK-like activity against K562 was significantly decreased in CHH individuals, compared with normals ($P < 0.001$, Fig. 1A). Thus, CHH individuals showed a markedly decreased specific CML, as well as NK-like cytotoxicity after 6 d of allogeneic culture, but showed normal spontaneous activity in the NK assay.

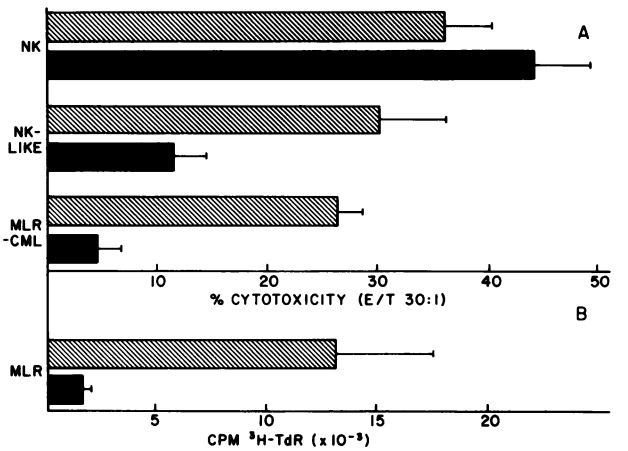

FIGURE 1 Cytotoxic mechanisms present in eight CHH (solid bars) and eight normal (striped bars) individuals. (A) Spontaneous NK cytotoxic activity was measured on day 0 against K562. Culture-induced nonspecific (NK-like, $P < 0.025$) and specific (MLR-CML, $P < 0.001$) cytotoxicity was measured after 6 d using K562 or MLR stimulator cells, respectively. (B) Allogeneic MLR-induced proliferation was assessed in microcultures after 5 d ($P < 0.005$).

Radiation sensitivity and the generation of radiation-resistant killing. Previous studies revealed that radiation sensitivity of spontaneous NK activity from healthy control subjects was under the control of an X-linked gene, with radiation resistance found in 10% of Caucasian subjects (15). NK-like and specific killing by MLR-generated effector cells prepared from radiation-sensitive subjects was found to be radiation resistant. Therefore, it was of interest to determine the relative ability of CHH effectors to develop radioresistance in allogeneic MLR cultures. Spontaneous NK activity was sensitive to irradiation in all CHH individuals tested, as well as in radiosensitive control subjects (data not shown). Culture-induced CML effectors from CHH individuals retained minimal cytotoxic activity after irradiation, compared with those from normal individuals, $P < 0.001$ (Table I). In contrast, the amounts of radiosensitive cytotoxicity of CHH and normal CML effectors were not significantly different. Similarly decreased radiation resistance was also found for NK-like activity against K562 induced in allogeneic cultures in CHH cells, compared with normal cells $P < 0.02$, (Table II). Radiation-sensitive NK-like activity was comparable in CHH and normal cells. The generation of radioresistant NK-like and specific cytotoxicity was correlated with allogeneic MLR-induced proliferation in seven CHH and normal individuals (proliferation vs. specific radioresistant cytotoxicity, CHH: $r = 0.94$, $P < 0.01$; normal: $r = 0.99$; $P < 0.01$; pro-

TABLE I

Radiation Resistance of CML Activity in CHH and Normal Individuals

CHH subject	Cytotoxic activity* Total	Radiation resistant‡	Radiation sensitive§	Normal subject	Cytotoxic activity* Total	Radiation resistant	Radiation sensitive
1	14.5	1.1	13.4	1	21.0	14.8	6.2
2	3.3	0	3.3	2	26.6	22.1	4.5
3	5.4	2.5	2.9	3	37.9	36.7	1.2
4	8.8	5.5	3.3	4	33.7	28.5	5.2
5	0	0	0	5	20.1	15.6	4.5
6	10.1	0	10.1	6	18.8	17.2	1.6
7	0	0	0	7	28.2	22.1	6.1
Mean±SEM	6.0±2.2	1.3±0.9	4.7±2.0		26.6±3.0	22.4±3.2	4.2±0.8
P‖	<0.001	<0.001	NS		—	—	—

* % ^{51}Cr release.

‡ Radiation-resistant cytotoxic activity is the cytotoxic activity remaining after effector cells have been irradiated with 3,000 rad.

§ Radiation-sensitive cytotoxic activity is the difference between the total cytotoxic activity minus the radiation-resistant activity.

‖ Comparison of CHH vs. normal by two-tailed unpaired Student's t tests.

liferation vs. radioresistant NK-like activity; CHH: $r = 0.88$, $P < 0.01$; normal: $r = 0.87$, $P < 0.05$).

Lymphocyte subpopulation analysis. It was of interest to relate the cytotoxic activities present in CHH PBMC to the lymphocyte subpopulations known to mediate cytotoxicity that are present in these individuals. In earlier work (4), we found a decrease in OKT3$^+$ (pan-T), OKT4$^+$ (helper/inducer), and OKT8$^+$ (suppressor/cytotoxic) T lymphocytes, normal proportions of $E_{AET}{}^+$ cells, and a marked increase in percentages of OKM1$^+$ lymphocytes in CHH. The monoclonal antibody analyses of PBL of the CHH and normal individuals in the present study is shown in Table III and are similar to our previous findings (4). OKT5$^+$ cells, reactive with a monoclonal antibody that identifies a subset of suppressor/cytotoxic T cells similar but not identical to the OKT8$^+$ subpopulation, were also decreased in CHH individuals ($P < 0.05$). E^+ cells were significantly lower, but $E_{AET}{}^+$ cells, which contain more low-affinity rosette-forming cells, were in normal range, in spite of a marked decrease in OKT3$^+$ cells in CHH patients, compared with controls.

In purified $E_{AET}{}^+$ cell fractions made from CHH PBMC, OKT3$^+$ cells were decreased ($P < 0.001$), and

TABLE II

Radiation Resistance of NK-like Activity in CHH and Normal Individuals

CHH subject	Cytotoxic activity* Total	Radiation resistant‡	Radiation sensitive§	Normal subject	Cytotoxic activity* Total	Radiation resistant	Radiation sensitive
1	16.2	2.3	13.9	1	13.6	3.2	10.4
2	5.0	1.2	3.8	2	36.5	30.6	5.9
3	13.4	11.2	2.1	3	49.5	45.0	4.5
4	9.3	7.1	2.2	4	22.2	5.9	16.3
5	4.5	0	4.5	5	23.8	15.6	8.2
6	26.7	2.7	24.0	6	16.2	3.7	12.5
7	4.9	0	4.9	7	17.5	14.7	2.8
Mean±SEM	11.4±3.3	3.5±1.7	7.9±3.3		25.6±5.3	17.0±6.4	8.7±1.9
P‖	<0.01	<0.02	NS		—	—	—

* % ^{51}Cr release.

‡ Radiation-resistant cytotoxic activity is the cytotoxic activity remaining after effector cells have been irradiated with 3,000 rad.

§ Radiation-sensitive cytotoxic activity is the difference between the total cytotoxic activity minus the radiation resistant activity.

‖ Comparison of CHH vs. normal by two-tailed unpaired Student's t tests.

TABLE III
Surface Marker Analysis of Subpopulations in CHH and Normal Individuals

Subjects	% positive peripheral blood lymphocytes*						
	OKT3	OKT4	OKT5	OKT8	OKM1	E‡	E_{AET}‡
CHH (n = 9)	52±7	36±5	11±4	18±3	44±7	69±2	87±2
Normal (n = 9)	77±3	55±2	30±3	32±3	18±2	79±1	88±1
P§	<0.005	<0.005	<0.05	<0.005	<0.001	<0.01	NS

* The absolute lymphocyte count, calculated from the WBC and differential, for CHH individuals was 1023±95/mm^3; normals 1952±183; $P < 0.001$.
‡ E rosettes were determined using untreated sheep erythrocytes; E_{AET} rosettes were determined using sheep erythrocytes pretreated with AET. Both were read following a 30-min incubation at 4°C.
§ Significance levels were determined by two-tailed unpaired Student's t tests.

both Fcγ^+ and OKM1$^+$ lymphocytes were significantly increased ($P < 0.05$, Table IV). In CHH individuals, there was a strong positive correlation between the increased percentages of Fcγ^+ cells and OKM1$^+$ cells ($r = 0.83$, $P < 0.01$). In normal subjects, Fcγ^+ and OKM1$^+$ cells represented only a small proportion of the total lymphocytes. In CHH, NK activity was significantly correlated with the number of OKM1$^+$ cells ($r = 0.80$, $P < 0.05$), as well as with the percentage of Fcγ^+ cells ($r = 0.74$, $P < 0.05$). In normal subjects, all NK activity does not appear to be mediated by OKM1$^+$ cells (11, 16). Thus, no significant correlations would be expected and none were detected.

Therefore, it appears that the subpopulation bearing an NK cell phenotype (Fcγ^+ OKM1$^+$ OKT3$^-$ OKT8$^-$ low affinity E$^+$) is proportionally increased in CHH individuals, whereas thymus derived OKM1$^-$ OKT3$^+$ OKT8$^+$ E_{AET}^+ cells, which mediate cytotoxic T-cell activity, are selectively depleted. These findings are consistent with the cytotoxicity studies in CHH in which NK activity is preserved while CML is markedly reduced.

Anti-K562 activity generated in CTCL. Analysis of CTCL confirmed the absence of culture-generated NK-like activity in CHH individuals. CTCL dependent on IL2 were established simultaneously from two normal and two CHH subjects. Both CHH CTCL showed diminished cytotoxicity against K562 targets when tested after 20 d of culture (Table V). No OKM1$^+$ or Fcγ^+ cells were detected in either CHH or normal CTCL cultures. Cells from both groups were greater than 90% E_{AET}^+ and OKT3$^+$. Both CHH and normal CTCL were ~50% OKT4$^+$ and 50% OKT8$^+$ and morphologically resembled 3-d phytohemaglutinin blasts. OKIa1 detected ~70% of the cells from both groups of individuals. As in allogeneic MLR-CML cultures, radioresistant cytotoxicity could not be generated in CHH CTCL (Table V).

DISCUSSION

Marked impairment of lymphocyte proliferation has been described in cartilage-hair hypoplasia (2–5). In

TABLE IV
Subpopulation Analysis of E_{AET} + Cells in CHH and Normal Individuals

Subjects	% positive E_{AET} + cells*		
	Fcγ‡	OKM1	OKT3
CHH (n = 9)	30±5§	38±1	70±6
Normal (n = 6)	14±2	12±1	92±1
P§	<0.05	<0.005	<0.001

* E_{AET}^+ cells were obtained after rosetting PBMC with AET-treated sheep erythrocytes.
‡ Fcγ rosettes were determined after overnight incubation with IgG-coated ox erythrocytes in serum-free media.
§ Significance levels were determined by unpaired two-tailed t tests.

TABLE V
Radiation Resistance of NK-like Cytotoxic Activity of CTCL from CHH and Normal Individuals

CTCL	Cytotoxic activity*		
	Total	Radiation resistant‡	Radiation sensitive§
CHH 1	4.2±0.4	1.5±0.1	2.7±0.4
CHH 2	4.7±0.2	0.7±0.1	4.0±0.2
Normal 1	21.0±2.1	17.2±0.3	3.8±2.1
Normal 2	39.9±1.1	27.4±1.6	12.5±2.6

* % ^{51}Cr release.
‡ Radiation-resistant cytotoxic activity is the cytotoxic activity remaining after effector cells have been irradiated with 3,000 rad.
§ Radiation-sensitive cytotoxic activity is the difference between the total cytotoxic activity minus the radiation-resistant activity.

this paper, we report that cellular cytotoxicity effector mechanisms are impaired but not constitutively absent. Culture-induced specific and NK-like cytotoxicity mediated by $OKT3^+$ T-cells is proliferation dependent, and was found to be absent or markedly diminished in CHH cultures. However, proliferation-independent spontaneous natural cytotoxicity is intact in CHH. Individuals with cartilage-hair hypoplasia appear to have a selective loss of high-affinity E-rosetting cells which are $OKT3^+$, whereas low-affinity E rosette-positive cells bearing the OKM1 and Fcγ receptor markers are preserved.

Spontaneous NK activity against K562 has been shown to be mediated by $Fc\gamma^+$ $OKM1^+$ $OKT3^-$ E^+ and E^- (null) large granular lymphocytes (LGL) (10–12, 17–19). In contrast, allogeneic MLR-induced specific cytotoxicity is mediated by the $OKT3^+$ $T5/T8^+$ T lymphocyte subpopulation (12–14), whereas the phenotype of the cells mediating NK-like culture-induced cytotoxicity has not been established. However, recent studies by Lopez-Botet et al. (20) suggest that NK-like activity is mediated by an IL2-responsive $OKT3^+$ T-cell blast. Seeley et al. (21) found that MLR-CML and NK-like against K562 were mediated by different cell types. They, and others, also reported that the anti-K562 culture-induced killing was not mediated by $Fc\gamma^+$ cells, in contrast to spontaneous NK activity (21–23). Our results support the concept that the cells mediating spontaneous NK activity are distinct from NK-like and MLR-CML, since (*a*) NK-like and specific cytolysis could not be generated in significant amounts in CHH individuals with impaired T cell function after culture, but NK activity was found in freshly isolated PBMC, and (*b*) $Fc\gamma^+$ and $OKM1^+$ cells decrease rapidly in MLR cultures, as culture-induced cytotoxicity increases, and are not present on CTCL, which bear strictly T-cell phenotypes ($OKT3^+T4^+$ or $OKT3^+T8^+$). Using clonally selected specific cytotoxic CTCL, others have also demonstrated the T cell nature of nonspecific anti-K562 cytotoxicity (24, 25). Moretta et al. (24) reported three out of four anti-K562 clones were $OKT8^+$ and all were E^+ and $Fc\gamma^-$.

The inability of CHH individuals to recruit $OKT5^+/T8^+$ cells to function in both HLA-restricted (MLR-CML) and nonrestricted killing (NK-like) is due, at least in part, to decreased production and utilization of interleukin 2 by CHH T cells (5). The clonal expansion of MLR-generated specific CML is dependent upon helper factors (IL2) synthesized by some $OKT4^+$ cells during the MLR sensitization (6, 13, 26). Radioresistant nonspecific cytotoxicity can be recruited by IL2-containing media (27). However, decreased IL2 production by CHH $OKT4^+$ cells cannot entirely explain the persistent radiosensitivity and severely depressed levels of anti-K562 activity by CHH CTCL, since excess exogenously supplied IL2 permits the recruitment of radioresistant effectors in normal lines, which themselves do not produce IL2. The failure of CHH PBMC and CTCL to develop culture-induced radioresistance supports the concept of defective recruitment due to an intrinsic defect in CHH T cells, which is also associated with decreased responsiveness to IL2 (5). In contrast, thymic-independent spontaneous NK activity does not require cell proliferation or soluble recruitment factors. Interestingly, the athymic nude mouse also has normal spontaneous NK activity and diminished culture generated cytolysis. However, *nu/nu* mice appear to have an isolated IL2 production deficit, and can generate cytolytic T cell effectors when exogenous IL2 is provided (28).

The in vivo role of spontaneous NK activity, as well as antibody-dependent cellular cytotoxicity, which is mediated by the same cell population, remains to be elucidated. Deficient spontaneous NK activity has been found in X-linked lymphoproliferative syndrome (29) and Chediak-Higashi syndrome (C-HS) (30). In both diseases, as well as in the beige mouse model of C-HS, the selective loss of NK effectors is associated with an increased susceptibility to infections and lymphoproliferative disorders (31). Only a minority of individuals with cartilage-hair hypoplasia have a history of severe viral infections (usually varicella, vaccinia, or polio) as well as recurrent, persistent respiratory infections, and in only a minority of these individuals is the outcome lethal (unpublished observations). Most CHH individuals are clinically healthy and survive well into adulthood despite profound in vitro cellular immune dysfunction (3, 4). Moreover, rates of lymphoproliferative and autoimmune diseases do not appear to be increased in CHH individuals (unpublished observations). Spontaneous natural cytotoxicity has been proposed to mediate host-defense against certain viral infections (i.e., vaccinia, cytomegalovirus, measles) (7–9), as well as immune surveillance against malignancies (31), and our studies of CHH support this theory. Our observations in CHH lend support to the importance of NK activity in human host defenses and raise questions regarding the relative importance of proliferation-dependent T cell mediated cytotoxic mechanisms. Thus, cartilage-hair hypoplasia provides a unique human model of lifelong partial cellular immunodeficiency for the analysis of the in vivo requirements for immunocompetence.

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