

# Deficiency of Active Natural Killer Cells in the Chediak-Higashi Syndrome

## LOCALIZATION OF THE DEFECT USING A SINGLE CELL CYTOTOXICITY ASSAY

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**ABSTRACT** This study investigated the defective natural killer (NK) cell activity in two patients with the Chediak-Higashi syndrome (CHS) using both a standard 51-chromium release microcytotoxicity and a single cell-in-agarose assay against K562 and Molt-4 target cells. CHS patients were deficient in overall maximum NK capacity, but had normal percentages of potentially cytotoxic target binding cells. The relative number of TBC that could kill bound targets (i.e., "active" NK cells) was significantly depressed in CHS patients when compared with normal controls. The diminished CHS active NK cells that were present, however, were capable of recycling and lysing multiple target cells during the assay period. In vitro interferon (INF) treatment of normal and CHS effector cells did not alter target cell binding, but did increase the maximum NK capacity, percentage of active NK cells, and the maximum recycling capacity, as well as the rate of lysis.

These studies indicate that the depression of NK activity in patients with CHS is secondary to a deficiency of active NK cells. The CHS active NK cells that are present, however, are capable of normal target lysis and recycling. Potentially cytotoxic pre-NK cells, which can bind but not kill target cells, can be activated by in vitro INF to develop lytic activity. Thus, INF treatment may be of potential benefit to the immune surveillance network of CHS patients by acti-

vating a population of pre-NK cells to express their cytotoxic potential.

### INTRODUCTION

The natural killer (NK)<sup>1</sup> cell system is believed to be an important effector limb of the immune surveillance network in animals and in man (1, 2). The in vivo relevance of this in vitro phenomenon in man has been suggested by several lines of evidence, included in which is the selective impairment in NK function in the Chediak-Higashi syndrome (CHS) (3, 4), a rare autosomal recessive disorder characterized by partial oculocutaneous albinism, severe recurrent pyogenic infections and abnormal lysosomal granules (5, 6). Patients who survive the infectious complications of the disease often succumb to an aggressive lymphoproliferative disorder later in life (7). The NK defect in these patients is not secondary to a lack of cells capable of recognizing and binding targets but appears to lie in the subsequent lytic process itself or in the recycling of NK cells.

To more precisely define the nature of the NK defect in CHS patients, our study was undertaken in which both a standard 51-chromium (51Cr) release microcytotoxicity assay and a single cell-in-agarose assay were used. Based on the methods of Grimm and Bon-

<sup>1</sup> *Abbreviations used in this paper:* CHS, Chediak-Higashi syndrome; 51Cr, 51-chromium; E/T, effector/target; FCS, fetal calf serum; INF, interferon;  $K_m$ , Michaelis constant; MRC, maximum recycling capacity; NK, natural killer; TBC, target binding cell;  $V_{max}$ , maximum NK capacity; V, number of killed targets.

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avida (8) and Bradley and Bonavida (9) for the measurement of cytolytic T cell activity, Ullberg and Jondal (10) have recently demonstrated that the percent target cell binding, the percentage of target binding cells that go on the lysing the target (i.e., "active" NK cells), and the particular NK capacity ( $V_{max}$ ) of a given heterogeneous population of effector cells can be quantitated by simultaneously using both of these assays. With these determinations, it is possible to then estimate the percentage of "active" NK cells and the number of target cells killed by a given NK cell, that is, the recycling capacity of a single cytotoxic cell (10). These methods have been extremely useful to date, particularly with regard to the enhancement of NK cell activity. For example, Silva et al. (11) have demonstrated that *in vitro* interferon (IFN) augments normal NK function by recruiting previously nonlytic "pre-NK" cells to display their cytotoxic potential as well as by enhancing the kinetics of lysis at the single effector-target cell level. Similarly, it has been shown that prostaglandin  $E_2$  (12) or moderate exercise (13) can synergistically interact with *in vitro* IFN to increase cytotoxicity by enhancing the recycling of active NK cells. Using  $^{51}Cr$  release microcytotoxicity and single cell assays simultaneously, we here report a deficiency of active NK cells in CHS patients, which can be partially corrected *in vitro* by IFN.

## METHODS

**Subjects.** Two brothers with CHS (LeR, age 29, and LaR, age 30) were studied on five separate occasions. LeR and LaR are the products of a consanguineous marriage, and their detailed clinical and immunologic histories have been extensively reported in the past (5, 6). At the time of this study, the patients were free of infection and were receiving no pharmacologic agents. Neither patient had any evidence of the progression of CHS into the lymphoproliferative disorder that characterizes this disease (7). Control subjects consisted of age- and sex-matched normal adults.

**Cell suspensions.** Ficoll-Hypaque-isolated peripheral blood mononuclear cells were depleted of adherent cells by passage through nylon wool columns (14) and were used as effector cells in all experiments. Nylon wool purification removed those adherent cells that might nonspecifically bind to target cells and produce a false estimate of the relative frequency of potentially cytotoxic NK cells. Nylon wool-purified lymphocytes contain <2% contaminating monocytes based on morphology and peroxidase staining and <2% surface immunoglobulin-bearing B cells. Cells were suspended in RPMI 1640 supplemented with 10–15% fetal calf serum (FCS).

**$^{51}Cr$  release assay.** A described  $^{51}Cr$  release microcytotoxicity assay (15) against the human erythroleukemia cell line, K562, and the human T cell line, Molt-4, was used. Briefly,  $10^6$  target cells were labeled for 1 h at  $37^\circ C$  with  $300 \mu Ci$  of  $^{51}Cr$  (ICN Nutritional Biochemicals, Cleveland, OH), washed three times, and resuspended in RPMI 1640 media with 10% FCS.  $10^4$  labeled target cells ( $100 \mu l$ ) were mixed with varying numbers of effector cells ( $100 \mu l$ ) in V-shaped microtiter wells (Flow Laboratories, Inc., Rockville,

MD) to give final effector/target (E/T) ratios of 100:1, 50:1, 20:1, and 5:1. Spontaneous release of  $^{51}Cr$  by target cells was determined by placing labeled target cells in microtiter wells in the absence of effector cells. Except as noted, cultures were incubated at  $37^\circ C$  in 5%  $CO_2$  in air at 100% humidity for 4 h. Plates were then centrifuged and  $100 \mu l$  of supernatant removed and counted in a gamma counter. Percent cytotoxicity (or percent  $^{51}Cr$  release) was determined by the formula: supernatant counts per minute minus spontaneous release counts per minute/total counts per minute minus spontaneous release counts per minute. In all experiments, spontaneous  $^{51}Cr$  release was <10%.

**Single cell-in-agarose assay.** This assay was performed by modification of the method of Ullberg and Jondal (10), based on the original descriptions of Grimm and Bonavida (8) and Bradley and Bonavida (9). Briefly,  $2 \times 10^5$  effector cells and unlabeled target cells were mixed in a total volume of 0.2 ml RPMI 1640 with 15% FCS in a 3-cm<sup>3</sup> round bottom tube. Using these conditions, no more than one lymphocyte is bound to any target cell, and therefore a true estimate of the frequency of effector-target cell conjugates can be obtained. At higher E/T ratios, several lymphocytes may bind to a single target cell, and therefore the percentage of conjugates cannot accurately be determined. Tubes were centrifuged at 500 g for 2 min and incubated at  $37^\circ C$  for 10–20 min followed by gentle resuspension a single time with a pasteur pipette. To optimize for the number of effector-target cell conjugates formed, this gentle resuspension technique was used because it has been demonstrated (9), and we have likewise observed that less avid effector-target cell conjugates can be disrupted by vigorous resuspension. By using this technique, we believe that we maximize the opportunity for these conjugates to remain intact. The cell mixture was then carefully added to 0.5 ml of 0.5% agarose in RPMI 1640 with 10 mM HEPES, which was precooled at room temperature from  $47^\circ$  to  $39^\circ C$ . Cells were mixed in agarose with a pasteur pipette and then poured onto 60-mm Petri dishes (Falcon Labware, Div. Becton, Dickinson & Co., Oxnard, CA) that had been precoated with  $0.5 \text{ cm}^3$  of 0.5% agarose. After the cell mixture in agarose had solidified, 6 ml of RPMI 1640 with 15% FCS was added, and the plate was incubated as above for 4 h, except as noted. After incubation, the media was removed, and 2 ml of 0.1% trypan blue was added for 10 min. Plates were then washed three times for 5 min each with cold phosphate-buffered saline and fixed with 1% formaldehyde, which removed all extracellular trypan blue.

The percentage of target binding cells (TBC), ~5–10% in normal subjects, was determined by counting the number of lymphocytes binding to target cells in 200–500 counted lymphocytes. The percentage of TBC with dead targets, normally 20–25% for K562 cells and 40–60% for Molt-4 cells, was determined by counting the number of dead targets in 100 effector-target conjugates. Spontaneous (or "background") target cell death was determined by counting the percentage of dead targets in the absence of effector cells. Corrections allowing for spontaneous target death are made by applying the following formula to calculate the percentage TBC with dead targets: (percentage of TBC with dead targets) minus (percentage of spontaneously dead targets) multiplied by (percentage of TBC with dead targets) (8–13).

**IFN-treatment.** In some experiments, effector cells were pretreated with IFN before the cytotoxicity assay.  $10 \times 10^6$  effector cells were incubated with 1,000 U human leukocyte IFN (generous gift of Dr. John J. Hooks obtained from Dr. K. Cantell, Karolinska Hospital, Stockholm, Sweden) or RPMI 1640 (control) for 30 min at  $37^\circ C$ , washed, counted,

and resuspended at the proper concentration. This IFN has been used in vivo in clinical trials and was derived from pooled human leukocytes exposed to ultraviolet light-inactivated viruses. This preparation is then purified by pH treatment and column fraction at which point it is suitable for in vivo use. The conditions selected for IFN treatment were those in which a maximal IFN-induced augmentation of both normal and CHS NK activity was observed. Using higher concentrations of IFN or longer incubation times did not significantly enhance normal or CHS NK activity to a degree greater than that observed with the conditions reported here.

**Analysis of data and statistical methods.** Calculations of cytotoxic functions were performed as outlined above and as described (10) for these assay systems. Data from the 51Cr release assay and the single cell assay were combined as done by Ullberg and Jondal (10) to determine particular  $V_{max}$  in a given effector population. Because the dose-response curve from 51Cr release assays resembles Michaelis-Menten enzyme-substrate kinetics, one can determine the  $V_{max}$  and Michaelis constant ( $K_m$ ) values (10). Ullberg and Jondal (10) have shown that  $V_{max}$  can be calculated from the Lineweaver-Burk equation:

$$\frac{1}{V} = \frac{K_m}{V_{max}} \times \frac{1}{T} + \frac{1}{V_{max}},$$

where T is the initial number of target cells and V the number of killed targets. As reported, experimental data obtained in this manner can be approximated by determining a 5:1 E/T ratio in the 51Cr release assay ( $5 \times 10^4$  effector cells and  $10^4$  target cells) and then using the formula:  $V_{max} = 1.4 \times 10^3 + 4.2 \times 10^2 \times (\text{percentage of cytotoxicity at 5:1})$ . In data not shown here, for normal subjects and CHS patients, the coefficient of correlation was  $>0.91$  in all cases when this formula was compared with the more tedious Lineweaver-Burk equation. Therefore, in data reported here the more simplified formula was used.

The percentage of "active" NK cells, that is, lymphocytes with bound and dead targets, was determined from the sin-

gle-cell assay by multiplying the percentage of TBC by the percentage of TBC with dead targets. This is  $\sim 1\text{--}2\%$  in normal subjects against K562 target cells and  $3\text{--}5\%$  against Molt-4 target cells. The maximum recycling capacity (MRC), which estimates the number of targets killed by an active NK cell in the 4-h assay, was determined by dividing  $V_{max}$  by the absolute number of active NK cells in the  $V_{max}$ . The MRC for normal subjects is  $\sim 5\text{--}6$  with K562 target cells and  $1\text{--}3$  with Molt-4 target cells.

Data were compared by the two-tailed Student's test.

## RESULTS

As described for these two patients (3, 4), NK activity against K562 and Molt-4 in a standard 51Cr release assay was markedly deficient at all E/T ratios when compared with normal controls (Fig. 1). Even with the assay time extended to 18 h, the cytotoxic function of CHS effector cells remained depressed (data not shown).

By using the 51Cr release assay and the single cell-in-agarose assay simultaneously, we were able to determine and compare the  $V_{max}$ , percent TBC, percent TBC with dead targets, percent active NK cells, and estimated MRC for normals and the CHS patients. As calculated from 51Cr release assay data, the CHS patients had significantly depressed values for  $V_{max}$  against K562 and Molt-4 targets when compared with normal subjects ( $P < 0.005$  for both patients for K562,  $P < 0.05$  for both patients for Molt-4) (Tables I and II). The percentage of CHS effector cells binding K562 and Molt-4 (i.e., TBC) in the single cell assay was not statistically different from normal individuals as has been described (3). However, CHS patients LaR and

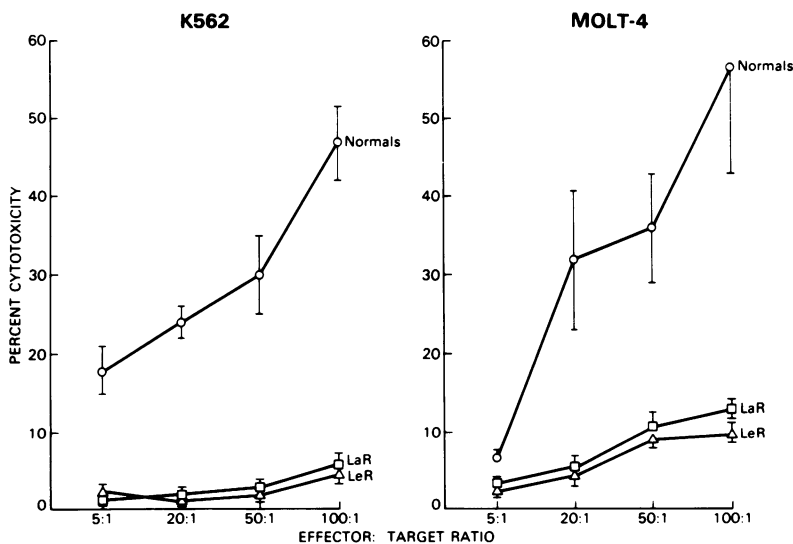


FIGURE 1 NK activity of normal and CHS (LaR and LeR) effector cells against K562 and Molt-4 target cells in a standard 4-h 51Cr release assay. Data represent the mean  $\pm$  SEM of three separate experiments for K562 and four separate experiments for Molt-4.

LeR were markedly deficient in the ability of their TBC to lyse either K562 or Molt-4 (Tables I and II). While normal NK cells lysed ~20% of bound K562, effector cells from both CHS patients killed only 5% of those conjugated targets ( $P < 0.001$  for both patients) (Table I). Similarly, while nearly 50% of bound Molt-4 cells were lysed by normal effectors, <20% of these attached targets were killed by CHS NK cells ( $P < 0.001$  for both patients) (Table II). This deficiency in lysis was present even when the assay time was extended to 18 h. (data not shown). Because the percentage of active NK cells is determined by both the percent TBC and percent TBC with dead targets, patients LaR and LeR had significantly reduced percentages of active NK cells against K562 ( $P < 0.005$  for both patients) and against Molt-4 ( $P < 0.001$  for both patients).

By using these data, it is possible to calculate the number of targets killed by a given NK cell in a 4-h assay; that is, the MRC estimates the number of times in 4 h that a single NK cell recognizes, binds to, lyses a target, and then repeats this process. This estimate is determined from the  $V_{max}$  and percentage of active NK cells as described in Methods (10). Although markedly deficient in both  $V_{max}$  activity and the percent-

age of active cytotoxic cells, NK cells from LaR and LeR had normal MRC values against both cell lines (Tables I and II).

It has been demonstrated (3) that NK activity of these CHS patients can be significantly augmented by *in vitro* IFN, but the level of cytotoxicity still remains significantly below that of non-IFN-treated normal effector cells. Because of this, we investigated the effects of *in vitro* IFN on the NK activity of normal and CHS lymphocytes in the currently used cytotoxicity assay systems. We selected Molt-4 as the target because relatively more bound Molt-4 cells are lysed compared with bound K562 targets. As shown in Table III, *in vitro* IFN increased the  $V_{max}$  of normal and CHS effector cells in the 4-h assay. The extension of the assay time to 6, 12, or 18 h did not significantly alter the values for normal or CHS NK cells activity from the levels observed at 4 h. Therefore, data are reported only for assay times up to 4 h. The percentage of normal and CHS TBC was not affected by IFN, but the percentage of TBC with dead targets was increased by IFN indicating that IFN induced previously non-lytic lymphocytes ("pre"-NK cells) to develop cytotoxic activity.

To determine the effects of *in vitro* IFN on the ki-

TABLE I  
NK Parameters of Normal Subjects and CHS Patients against K562 Target Cells

Subject	$V_{max}$ $\times 10^6$	TBC %	TBC with dead K562 targets %	Active NK cells %	Estimated MRC
<b>Normals</b>					
Exp. 1	11.5	7.5	25	1.9	6.5
Exp. 2	8.1	6.5	21	1.4	5.8
Exp. 3	7.7	6.5	22	1.4	5.5
Mean $\pm$ SEM	9.1 $\pm$ 1.2	6.8 $\pm$ 0.3	22.7 $\pm$ 1.2	1.6 $\pm$ 0.2	5.9 $\pm$ 0.3
<b>LaR</b>					
Exp. 1	1.6	7.5	5.0	0.38	4.2
Exp. 2	1.8	10	3.2	0.32	5.7
Exp. 3	1.6	7	5.9	0.41	4.0
Mean $\pm$ SEM	1.7 $\pm$ 0.07 ( $P < 0.005$ )*	8.2 $\pm$ 0.9 ( $P = NS$ )†	4.7 $\pm$ 0.8 ( $P < 0.001$ )	0.37 $\pm$ 0.03 ( $P < 0.005$ )	4.6 $\pm$ 0.5 ( $P = NS$ )
<b>LeR</b>					
Exp. 1	2.0	8	4.3	0.34	5.9
Exp. 2	1.8	7	5.3	0.37	4.9
Exp. 3	1.9	8	5.1	0.41	4.6
Mean $\pm$ SEM	1.9 $\pm$ 0.05 ( $P < 0.005$ )	7.7 $\pm$ 0.3 ( $P = NS$ )	4.9 $\pm$ 0.3 ( $P < 0.001$ )	0.37 $\pm$ 0.02 ( $P < 0.005$ )	5.1 $\pm$ 0.4 ( $P = NS$ )

\*  $P$  values compared to normals.

†  $P$  value not statistically significant.

TABLE II  
NK Parameters of Normal Subjects and CHS Patients against Molt-4 Target Cells

Subject	Vmax	TBC	TBC with dead Molt-4 targets	Active NK cells	Estimated MRC
	$\times 10^6$	%	%	%	
<b>Normals</b>					
Exp. 1	6.0	7	55	3.9	1.5
Exp. 2	5.2	9	42	3.8	1.4
Exp. 3	14.8	12	40	4.8	3.1
Exp. 4	7.4	7	55	3.9	1.9
Mean $\pm$ SEM	8.4 $\pm$ 2.2	8.8 $\pm$ 1.2	48 $\pm$ 4.1	4.1 $\pm$ 0.2	2.0 $\pm$ 0.4
<b>LaR</b>					
Exp. 1	2.7	5.5	18	0.99	2.7
Exp. 2	2.7	7.5	19	1.4	1.9
Exp. 3	1.9	7	21	1.5	1.3
Exp. 4	1.6	6.5	14	0.91	1.8
Mean $\pm$ SEM	2.2 $\pm$ 0.3 ( $P < 0.05$ ) <sup>*</sup>	6.6 $\pm$ 0.4 ( $P = \text{NS}$ ) <sup>†</sup>	18 $\pm$ 1.5 ( $P < 0.001$ )	1.2 $\pm$ 0.2 ( $P < 0.001$ )	1.9 $\pm$ 0.3 ( $P = \text{NS}$ )
<b>LeR</b>					
Exp. 1	3.5	9	16	1.4	2.5
Exp. 2	3.6	10	19	1.9	1.9
Exp. 3	1.9	8.5	23	2.0	0.95
Exp. 4	2.0	6.5	14	0.91	1.8
Mean $\pm$ SEM	2.8 $\pm$ 0.5 ( $P < 0.05$ )	8.5 $\pm$ 0.7 ( $P = \text{NS}$ )	18 $\pm$ 2.0 ( $P < 0.001$ )	1.6 $\pm$ 0.3 ( $P < 0.001$ )	1.8 $\pm$ 0.3 ( $P = \text{NS}$ )

\*  $P$  value compared to normals.

†  $P$  value not statistically significant.

netics of lysis, normal, and CHS effector cells were assayed in 51Cr release and single-cell assays at 1, 2, 3, and 4 h after IFN treatment and mixture with Molt-4 target cells. At each time point, both the percentage of TBC with dead targets and the percentage of active NK cells increased in both the normal subject and the CHS patients (Fig. 2). That is, for control and CHS subjects, a given level of either of these two cytotoxicity parameters was achieved earlier when effector cells were treated with IFN, indicative of an increased rate of killing.

## DISCUSSION

This study has clearly demonstrated that the overall deficiency of NK activity in CHS patients is secondary to an inability of potentially cytotoxic lymphocytes capable of binding target cells to complete the lytic sequence. As described, the percentage of CHS TBC is comparable to normal values (3). Because these TBC populations contain lymphocytes with NK activity and because target binding is a prerequisite for lysis to

occur, the lytic defect in CHS patients was not secondary to a failure of CHS NK cells to recognize or bind to susceptible target cells. However, within the population of CHS TBC there was a marked deficiency of active NK cells. These CHS active NK cells that were present, however, were not only capable of killing their bound targets but could repeat the entire lytic sequence (i.e., "recycle") in a manner comparable to normals. Thus, these active CHS effector cells appeared to be normal in all phases of the lytic event: target cell recognition and binding, lysis of the bound target cell, detachment from the dead target, and recycling.

In vitro IFN increased normal and CHS NK activity as determined by several different parameters. The overall Vmax of normal and CHS effector cells was increased by in vitro IFN. Because this value is directly calculated from 51Cr release microcytotoxicity values, this would be expected given the IFN-induced increase in 51Cr release from target cells. Similar findings for overall CHS NK activity have been reported using this same assay system (3).

TABLE III  
The Effect of In Vitro IFN on NK Parameters of Normal Subject and CHS Patients against Molt-4 Target Cells

		DV (normal)	LaR	LeR
Vmax, $\times 10^3$	Control*	8.4	2.6	2.0
	IFN*	14.8	5.6	4.9
TBC, %	Control	7	6.5	6.5
	IFN	8	7	6.5
TBC with dead targets, %	Control	57	15	14
	IFN	75	27	31
Active NK cells, %	Control	4.0	1.0	0.9
	IFN	6.0	1.9	2.0
Estimated MRC	Control	2.1	2.6	2.1
	IFN	2.5	3.0	2.5

\* Effector cells were treated with media (control) or IFN (1,000 U/10  $\times 10^6$  lymphocytes) for 30 min at 37°C before the assay.

In vitro IFN also increased the percentage of active NK cells indicative of IFN induction of previously nonlytic pre-NK cells to develop cytotoxic activity. Comparable findings with normal lymphocytes have been recently reported using similar single-cell cytotoxicity assays (11–13, 16). This is reflected in the percentages of normal and CHS active NK cells in control and IFN-treated populations. Although the levels of CHS TBC with dead targets and active NK cells still remained depressed compared with normal values, the relative IFN-induced increase in these parameters for CHS effector cells was greater than that observed with normal lymphocytes. That is, the percentage of CHS TBC with dead targets doubled after IFN treatment, while values for the normal subject increased by approximately one-third (Table III). Similarly, in vitro IFN increased the percentage of CHS active NK cells twofold, while increasing this parameter in the normal control by only 50% (Table III). However, the absolute increase in the percentage of active NK cells (2% for normal, 1% for CHS patients) was essentially the same, and the greater relative increase in CHS active NK cells may have resulted from a fewer number of active NK cells before IFN treatment.

In vitro IFN may have also increased CHS and normal NK activity by augmenting effector cell recycling. IFN slightly enhanced the NK MRC of normal and CHS effector cells, which were comparable before and after IFN treatment. The values for MRC after IFN are suggestive of an IFN-induced increase in this parameter as described using these same assays (10–13, 16). However, because the overall values for normal and CHS MRC are low as are the relative increments in this parameter, additional study is necessary to further document the significance of this finding.

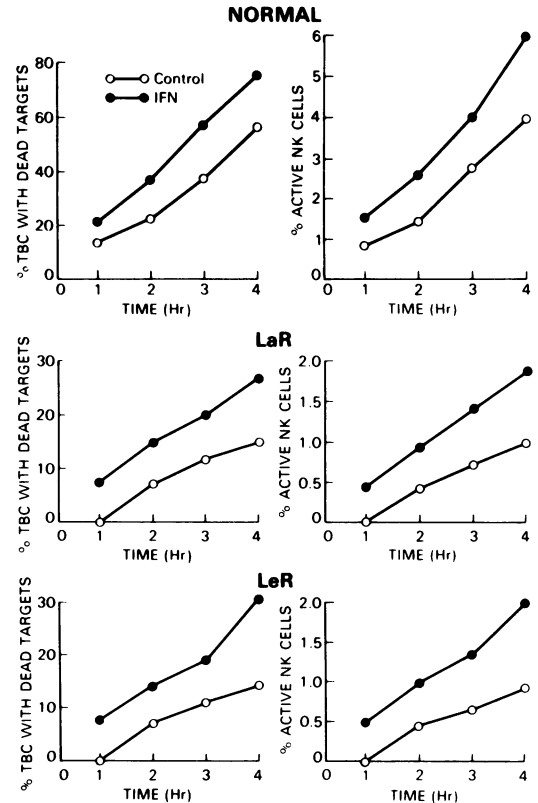


FIGURE 2 The effect of in vitro IFN on NK function of normal and CHS (LaR and LeR) effector cells against Molt-4 target cells in the single-cell cytotoxicity assay. Lytic activity was determined at 1, 2, 3, and 4 h after the initiation of the assay. Peak cytotoxic activity was observed with a 4-h assay time and prolongation of the assay for 6, 12, or 18 h did not significantly differ from the cytotoxic values observed at 4 h. Left panels indicate the percentage of TBC with dead targets at each time point. Right panels show the data as the percentage of active NK cells (percent TBC  $\times$  percent TBC with dead targets) at each time point.

An additional mechanism of the IFN-induced increase in normal and CHS NK activity is an alteration in the kinetics of lysis. For normal and CHS effector cells, in vitro IFN increased the percentage of TBC with dead targets and the percentage of active NK cells at each time point assayed, indicative of an increased rate of lysis as described for normal lymphocytes (16). These studies, therefore, demonstrate that in vitro IFN augments the deficient CHS NK activity in exactly the same manner as it enhances normal NK function, i.e., the overall NK capacity of a given population of NK cells (Vmax) is increased secondary to (a) an activation of previously noncytotoxic pre-NK cells, (b) a slight increase in the MRC of NK cells, and (c) an increase in the rate of lysis.

These studies have localized the defect in CHS NK activity to a deficiency of active NK cells. Those CHS

active NK cells that are present, however, behave normally in all respects. Because the percentage of TBC in CHS patients is normal, the defect must lie in the activation of nonlytic pre-NK cells to express their cytotoxic potential. These pre-NK cells can bind to but not lyse target cells, suggesting that they bear specific antigen receptors. The mere binding of target cells to these receptors then is not sufficient for cytotoxic function to be expressed. Additionally, different cell lines appear to vary in their ability to activate pre-NK cells (10). Although we did not use other target cells that might be lysed by different mechanisms, the NK defect in CHS patients appears to be consistent regardless of the target system used (3). Because K562 and Molt-4 target cells are among the more NK-sensitive target cells, we chose to limit our study to these cell lines.

The percentage of normal and CHS NK cells activated by K562 targets is far less than the percentage activated by Molt-4 target cells. Therefore, additional factors other than simple target binding are necessary for the full cytotoxic potential to be expressed by NK cells. In vitro IFN, which increases the percentage of active NK cells, may do so by providing the extra "signal" needed for some pre-NK cells to express their lytic ability. Because CHS patients have well documented defects in microtubule function, which in turn lead to an abnormality in the release of lysosomal enzymes (12), one can postulate that full expression of lytic activity and the activation of pre-NK cells to become active NK cells is in some manner dependent upon intact microtubule function. However, further studies are obviously needed to substantiate this hypothesis.

These studies indicate that there exists a population of CHS lymphocytes that is capable of normal NK activity, that is, target cell recognition and binding, target cell lysis, and recycling. CHS patients are relatively deficient in this population of active NK cells when compared with normal subjects. That there are normal numbers of CHS TBC, which are in theory at least potentially cytotoxic, and that IFN activates some of these cells to become lytic, would suggest that there are some pre-NK cells in CHS patients in which NK activity can be induced by the proper stimulus.

Thus, the defect in CHS NK activity is secondary to a deficiency of active NK cells, and this deficiency can be partially corrected by the activation of pre-NK cells by the appropriate stimulus. Those CHS lymphocytes that have normal base-line lytic activity appear to be entirely normal with regard to all events in the lytic sequence and in the response to in vitro IFN. The development of lymphoproliferative disorders in CHS patients suggests that this tendency may be secondary to a deficiency of active NK cells capable of normal immune surveillance. Because in vitro IFN

partially corrects this defect, it is conceivable that in vivo IFN therapy may benefit these patients by activating quiescent pre-NK cells to express their cytotoxic potential.

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#### REFERENCES

1. Heberman, R. B., and H. T. Holden. 1978. Natural cell-mediated immunity. *Adv. Cancer Res.* 27: 305-377.
2. Heberman, R. B., J. Y. Djeu, D. H. Kay, J. R. Ortaldo, C. Riccardi, G. D. Bonnard, H. T. Holden, R. Santoni, A. Santoni, and P. Pucetti. 1979. Natural killer cells: characteristics and regulation of activity. *Immunol. Rev.* 44: 43-70.
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. R. 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. Blume, R. S., and S. M. Wolff. 1972. The Chediak-Higashi syndrome: studies in four patients and a review of the literature. *Medicine (Baltimore).* 51: 247-280.
6. Root, R. K., A. S. Rosenthal, and D. J. Balestra. 1972. Abnormal bactericidal, metabolic, and lysosomal functions of Chediak-Higashi syndrome leukocytes. *J. Clin. Invest.* 51: 649-665.
7. Dent, P. B., L. A. Fish, J. F. White, and R. A. Good. 1966. Chediak-Higashi syndrome. Observations on the nature of the associated malignancy. *Lab. Invest.* 15: 1634-1642.
8. Grimm, E., and B. Bonavida. 1979. Mechanism of cell-mediated cytotoxicity at the single cell level. I. Estimation of cytotoxic T lymphocyte frequency and relative lytic efficiency. *J. Immunol.* 123: 2861-2868.
9. Bradley, T. P., and B. Bonavida. 1981. Mechanism of cell-mediated cytotoxicity at the single cell level. III. Evidence that cytotoxic T lymphocytes lyse both antigen-specific and -nonspecific targets pretreated with lectins or periodate. *J. Immunol.* 126: 208-213.
10. Ullberg, M., and M. Jondal. 1981. Recycling and target binding capacity of human natural killer cells. *J. Exp. Med.* 153: 615-628.
11. Silva, A., B. Bonavida, and S. Targan. 1980. Mode of action of interferon-mediated modulation of natural killer cytotoxic activity: recruitment of pre-NK cells and enhanced kinetics of lysis. *J. Immunol.* 125: 479-484.
12. Targan, S. R. 1981. The dual interaction of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) and interferon (IFN) on NK lytic activation: enhanced capacity of effector-target lytic interactions

- (recycling) and blockage of pre-NK cell recruitment. *J. Immunol.* **127**: 1424-1428.
13. Targan, S., L. Britvan, and F. Dorey. 1981. Activation of human NKCC by moderate exercise: increased frequency of NK cells with enhanced capability of effector-target lytic interactions. *Clin. Exp. Immunol.* **45**: 352-360.
  14. Julius, M. H., E. Simpson, and L. A. Herzenberg. 1973. A rapid method for the isolation of functional thymus-derived murine lymphocytes. *Eur. J. Immunol.* **3**: 645-649.
  15. Katz, P., C. B. Simone, P. A. Henkart, and A. S. Fauci. 1980. Mechanisms of antibody-dependent cellular cytotoxicity. The use of effector cells from chronic granulomatous disease patients as investigative probes. *J. Clin. Invest.* **65**: 55-63.
  16. Targan, S., and F. Dorey. 1980. Interferon activation of "pre-spontaneous killer" (pre-SK) cells and alteration in kinetics of lysis of both "pre-SK" and active SK cells. *J. Immunol.* **124**: 2157-2161.
  17. Oliver, J. M. 1976. Impaired microtubule function correctable by cyclic GMP and cholinergic agonists in the Chediak-Higashi syndrome. *Am. J. Pathol.* **85**: 395-412.