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Susceptibility of Human Leukaemias to Cell-Mediated Cytotoxicity by Interferon-Treated Allogeneic Lymphocytes

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Summary. *Twenty-two human leukaemias, comprising acute phase leucocytes from 13 acute myeloid and nine lymphoid leukaemias, were tested for susceptibility to spontaneous cell-mediated cytotoxicity (CMC) by untreated lymphocytes and lymphocytes treated for 18 h with 250 IU lymphoblastoid (Namalva) interferon (IFN-α). IFN-amplified killing (IAK) by lymphocytes from 24 normal lymphocyte donors was checked on the K562 erythroleukaemia cell line, for comparison with IAK on fresh leukaemias. Nine leukaemias were tested with lymphocytes from three donors, nine with lymphocytes from six donors, three with lymphocytes from nine donors, and one with lymphocytes from 11 donors. Some degree of susceptibility to IAK was found in five acute myeloid and five lymphoid leukaemias, which was markedly dependent upon the source of the effector lymphocytes and did not correlate with the degree of IAK on K562. The 12 other leukaemias were virtually resistant to IAK. The results emphasize the variability in the capacity of IFN-treated lymphocytes to lyse leukaemias that have not been adapted to tissue culture. The basis of effector recognition of cell line and fresh tumour targets is discussed.*

Introduction

Human natural cell-mediated cytotoxicity is exhibited by a population of lymphoid cells, possibly of T lineage [10, 11, 24, 36], bearing Fc receptors [2, 12], whose greatest activity is found in the spleen and peripheral blood [20] and which are usually referred to as natural killer (NK) cells.

Unlike cytotoxic T lymphocytes (CTL), NK cells appear to lack immunological memory and histocompatibility restriction, but show intraspecies preference and exhibit considerable variation according to age, sex, and genetic background [see reviews by Herbermann et al.: 6, 7].

Whilst a range of target cells, including tumour and non-tumour lines, are lysed by NK cells, most studies have employed targets adapted to tissue culture. Freshly harvested human tumours, including leukaemias [16, 27] and lung cancer cells [35] show greater resistance to NK-CMC than cultured cell-lines (CCL). Nevertheless, the susceptibility of CCL from tumours observed by some authors [9], and that of normal neonatal thymocytes and foetal bone marrow cells to NK-CMC [5], suggests that natural cell-mediated immunity may have an important systemic role in the control of certain cellular interactions of biological significance. However, the nature of the target cell structure(s) recognized by NK cells is unknown. The variable sensitivity of different targets, against which peripheral blood NK cells (PBL-NK) are commonly assayed, does not correlate with any of the known cell surface antigens [26].

Previous attempts to demonstrate cellular cytotoxicity to fresh human leukaemias showed that levels were at best rather low [16, 22, 27], though results are likely to have been affected by disease status and therapy. Rosenberg et al. [27] showed that lymphocytes from identical twins of leukaemia patients were unequivocally cytotoxic against the patient's leukaemia, which is possibly the most cogent evidence of a population of cytotoxic lymphocytes with reactivity against fresh leukaemias.

Several studies have shown that lymphocytes stimulated in vitro with different types of allogeneic cell can lyse autologous and HLA-identical leukaemias [8, 28, 30, 31, 37], but the relationship between this cytotoxicity and that attributable to NK cells is not clear. Recently, we reported that the susceptibility of several human T cell leukaemia cell lines frequently used in NK assays could be enhanced by pretreatment of PBL with exogenous interferon (IFN) [19]. Although the interferon-amplified killing (IAK) appeared to be mediated by cells with similar properties to PBL-NK, they exhibited some differences, notably activity against a wider range of targets, including NK-resistant ones. This broader spectrum of lytic activity associated with lymphocytes activated in vitro under a variety of conditions [18, 25, 29] prompted us to determine to what extent fresh, non-cultured human leukaemias, to date largely NK-resistant in our hands and those of other investigators [23, 27], might be rendered susceptible to IAK.

Materials and Methods

Target Cells. Leukaemia cells obtained from patients prior to treatment were separated from peripheral blood and cryopreserved as previoulsy described [31]. The differential leucocyte counts of freshly thawed leukaemias and the diagnostic types upon bone marrow examination are given in Table 1.

The cell line K562 derived from a Ph^+ve chronic myeloid leukaemia patient in blast crisis [17], but recently reclassified as an erythroleukaemia [1], was used to monitor CMC by untreated and interferon-treated peripheral blood lympho-

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Table 1. Differential white cells counts of human leukaemias

Cell code	Diagnosis	Blasts	Lymphs	Percentage cells				
				Promyelo	Myelo/meta	Neutr	Mono	Eo/Baso
E69	AML	86	12			2		
E70	AMML	NA[d]						
E71	AML	69	30		1			
E72	AML	95	4		0	1	0	0
E73	AMML	77	18			3	1	1
E75	AML	88	9	1	2			
E76	AML	80	17	2		1		
E77	ALL	79	21					
E78	ALL	68	13			16	3	
E79	ALL	77	23					
E81	ALL	90	10					
E82	AML	NA						
E83[a]	CML-ALL	76	24					
E84	AMML	82	18					
E85	AMML	75	15		4		6	
E86[b]	Lym.	88	9					
E87	AMML	86	12	2			6	
E88	AML	81	12		1			
E89	ALL	80	20					
E90	AML	82	16		2			
E91	ALL	76	24					
EJu[a]	Ph^+CML BlCr	82	10		2	6		

[a] Ph^+ve CML transformed to ALL
[b] Diffused immunoblastic lymphoma
[c] Abbreviations: AML, acute myeloblastic leukaemia; AMML, acute myelomonocytic leukaemia; ALL, acute lymphoblastic leukaemia
[d] Not available

cytes (PBL). It was grown in suspension culture in HEPES-buffered RPMI-1640 containing 10% heat-inactivated foetal calf serum (RPMI-FCS) and antibiotics.

Interferon (IFN). Human lymphoblastoid IFN-α was prepared and purified by Dr K. H. Fantes, Wellcome Research Laboratories, Kent, England. The preparation (batch 479/602) used in this study was of specific activity 2.2×10^6 IU/mg protein. The units refer to a British Standard Unit calibrated against Std B69/19 (National Institute of Biological Standards Controls, London, England). The IFN, which contained added human plasma protein as a stabilizer, was stored at −70° C. Before use, freshly thawed IFN was diluted with RPMI-FCS.

Preparation and Culture of Human Leucocytes. Heparinised blood from each of 24 normal donors aged between 24 and 60 years was separated as previously described [24]. Mononuclear cells used as effectors routinely contained > 85% lymphocytes. The leucocytes (3×10^6/ml in RPMI-FCS) were cultured in 250 IU · ml^{-1} IFN at 37° C in humidified 5% CO_2 air for 18 h. Under these conditions CMC to K562 by PBL is optimally amplified [19]. After 18 h the leucocytes were washed three times in HBSS, counted and checked for viability, then diluted to the appropriate concentration in RPMI-FCS. Leucocytes for control tests were incubated for the same time (18 h) but without IFN, and thereafter treated in the same way as the IFN-treated leucocytes.

Cell-Mediated Cytotoxicity (CMC) Assay. The CMC assay was a modification of that described by Potter and Moore [24]. Target cells, labelled for 1 h with ^{51}Cr (Radiochemical Centre, Amersham Bucks.) were washed three times and 100-µl aliquots (10^4 cells) were each mixed with 100 µl effector cells in microplates with 96, round-bottomed, wells (M24 ART, Sterilin, UK). Contact between effector and target cells was promoted by centrifugation at 60 *g* for 1 min. Effector-to-target (E : T) ratios of 40 : 1, 20 : 1, and 10 : 1 were routinely used. In some tests ratios of 80 : 1 and 140 : 1 were used with fresh leukaemia targets, but only 20 : 1 was used for the K562 cell line. Spontaneous release of ^{51}Cr was determined in wells containing target cells only. Maximum ^{51}Cr release was assessed by addition of Triton X100 (1/100 dilution) to target cells alone. Each effector : target combination was set up in triplicate, after which the plates were incubated for 6 h at 37° C in 95% air: 5% CO_2 and centrifuged again (5 min); 100-µl samples of the supernatants were then removed and counted on a Searle 1185 gamma-counter. Percentage ^{51}Cr release was determined for each well and the mean value of triplicate wells was used to calculate the percentage cytotoxicity according to the following formula:

$$\text{Percentage cytotoxicity} = \frac{(\%\ ^{51}\text{Cr release by effectors} - \%\ ^{51}\text{Cr spontaneous release}}{(\%\ ^{51}\text{Cr total release} - \%\ ^{51}\text{Cr spontaneous release}} \cdot$$

Spontaneous ^{51}Cr release during 6 h for K562 was 5%−15% and for the leukaemias, 6%−35%. Negative cytotoxicity denotes that ^{51}Cr release from target cells alone exceeded that from target cells in the presence of effectors. The standard error of triplicates was < 5%.

Table 2. Normal and interferon-amplified killing of allogeneic acute leukaemias and K562 cells

Leukaemia Target	E : T Ratio	Donor					
		4		5		6	
		−IFN	+IFN[c]	−IFN	+IFN[c]	−IFN	+IFN[c]
E77	40 : 1	9.3	40.9	− 2.6	7.5	0	15.9
(12.2)[b]	20 : 1	5.9	31.1	− 0.7	2.3	− 1.3	11.3
	10 : 1	2.9	22.1	0.6	4.3	6.8	9.8
E79	40 : 1	7.5	21.8	1.6	8.4	0.6	2.6
(21.3)[b]	20 : 1	5.6	11.7	1.9	5.9	2.3	2.8
	10 : 1	3.2	9.5	3.4	7.1	0	1.8
E81	40 : 1	12.1	15.0	− 3.7	9.4	6.4	10.0
(35.0)[b]	20 : 1	9.3	12.1	3.6	7.8	0.3	4.5
	10 : 1	NT	NT	2.1	2.4	6.2	4.4
E82	40 : 1	− 4.6	− 0.2	− 4.5	5.8	−12.9	− 5.3
(32.0)[b]	20 : 1	− 3.6	− 3.8	− 8.0	9.4	− 8.5	− 4.5
	10 : 1	− 7.4	− 3.1	− 6.8	− 9.1	−10.4	0
K562	20 : 1	22.6	49.5	40.5	71.9	2.5	19.0
(14.4)[b]							

[a] ALL, E77, E79 and E81; AML, E82.
[b] Spontaneous isotope release (%) in parantheses NT = Not tested
[c] Pretreatment: 250 IU IFN-α ml^{-1} for 18 h

Results

Cell-mediated cytotoxicity (CMC) on the 22 leukaemias by IFN-treated and untreated lymphocytes from 24 unrelated normal donors was determined in eight experiments. Each leukaemic target cell was tested with effectors from a minimum of three and a maximum of 11 donors, in parallel with tests on K562 cells. Nine leukaemias were tested with three donors, nine with six donors, three with nine donors and one with 11 donors.

Our experience with K562 had shown that CMC by many normal donor lymphocytes is already high at an E : T ratio of 40 : 1 and that IFN has relatively little amplifying effect. CMC on K562 cells in these experiments was therefore routinely assayed at an E : T ratio of 20 : 1. By contrast, preliminary experiments showed that fresh leukaemias were generally more resistant than K562 to CMC. The highest E : T ratio in tests involving leukaemic targets was therefore routinely adjusted to 40 : 1 and in some cases increased to 80 : 1 and 140 : 1.

Data from a single representative experiment (Expt 2) in which four leukaemias (3 ALL, 1 AML) were tested against a panel of three lymphocytes (donors 4, 5 and 6) are given in Table 2. CMC on the leukaemias was demonstrable in two (E77, E79) of the four tested, as judged by evidence of (i) amplification of cytotoxicity by IFN-treated effectors, compared with untreated PBL; and (ii) a dose-response relationship over the three E : T ratios tested. The apparently weak susceptibility of a third leukaemia, E81, was only marginally amplified by IFN-activated lymphocytes.

CMC data on K562 and ten leukaemias are summarized in Table 3. Considerable variation in the level of IFN-induced amplification was observed against both the cell line (at E : T ratio 20 : 1) and the fresh targets (at E : T ratio 40 : 1). The results of the tests on ten leukaemias including five myeloid (E72, E76, E84, E85, and E87) and five lymphoid (E77, E79, E81, E86, and E89) in Table 3 comprise values in which CMC against the leukaemia targets was > 10% with lymphocytes from at least one donor, and in which cytotoxicity followed the dose-response pattern characteristic of cell-line killing. The widest differences between IAK and unstimulated CMC were found on E72 (25%), E76 (29%), and E84 (31%) among the myeloid leukaemias, and E77 (32%) and E79 (29%) among the lymphoid leukaemias. However, the level of IAK on the leukaemias was almost without exception lower than that on K562 cells.

Whereas all donor lymphocytes killed K562 cells, to a greater or lesser degree, susceptibility of the leukaemias (e.g., E72, E87, E89) was confined to a minority, and then only after IFN activation. Furthermore, it was apparent that a given donor exhibiting potent amplification of CMC by IFN on K562 cells might be relatively weakly cytotoxic on a panel of leukaemias (e.g., donor 1 vs E84, E85, E87, and E79).

Of the remaining leukaemias depicted in Table 1 and not shown in Table 3, all were judged to be resistant since they gave CMC values consistently < 10% and no evidence of amplification by IFN (e.g., E82, Table 2).

The results of tests on all leukaemias are summarized in Table 4, where details are given of the number of donors tested on each leukaemia, in comparison with the number of positive CMC tests with and without IFN treatment of the effector cells.

The possibility that cytotoxicity absent at an E : T ratio of 40 : 1 could have been detected at E : T ratios of 80 : 1 and 140 : 1 was examined with several of the 'resistant' leukaemias, with consistently negative results. Allogeneic PHA-transformed lymphoblasts tested with untreated and IFN-treated effector cells were also totally resistant to CMC (data not shown).

The results summarized in Table 4 show that only one of the 22 leukaemias gave positive CMC (> 10%) with one or more donor lymphocytes without IFN pretreatment, compared

Table 3. Increase in CMC (%) by interferon-treated lymphocytes in different leukaemias[a]

Donor no.	Cell line K562[b]	Acute myeloid leukaemias				
		E72	E76	E84	E85	E87
1	44.2 (53.3)			13.8 (17.9)	0.4 (2.2)	5.3 (5.4)
2	42.0 (60.2)			11.4 (18.2)	6.6 (7.2)	5.9 (7.7)
3	55.5 (69.2)			7.1 (7.0)	4.5 (6.1)	0 (−1.6)
4	26.9 (49.5)					
5	31.4 (71.9)					
6	16.5 (19.0)					
7	7.0 (13.7)	0 (0)				
8	8.0 (35.4)	1 (2.7)				
9	14.8 (33.8)	25.4 (31.9)				
10	23.2 (30.9)		4.5 (4.5)			
11	22.3 (59.8)		15.1 (17.0)			
12	27.3 (59.6)		21 (25.9)			
13	7.9 (32.8)	3.4 (5.6)				
14	8.5 (14.4)	0 (0)				
15	15.3 (30.4)	0 (0)				
16	26.0 (27.3)		28.6 (28.6)	33.5 (40.3)	11.2 (13.2)	18.9 (25.6)
17	20.4 (29.0)		21.5 (21.5)	19.3 (26.9)	0.9 (6.1)	14.4 (21.0)
18	26.9 (31.6)		0 (0)	9.1 (10.9)	1.3 (1.3)	4.7 (6.2)
19	TF					
20	22.9 (29.6)					
21	22.5 (28.4)					
22	11.6 (19.6)					
23	10.4 (21.3)					
24	9.6 (16.4)					

Donor no.	Cell line K562[b]	Acute lymphoid leukaemias				
		E77	E79	E81	E86	E89
1	44.2 (53.3)		4.3 (5.5)	0 (−6.0)	3.2 (13.7)	
2	42.0 (60.2)		0 (0.9)	0 (−7.3)	6.6 (12.6)	
3	55.5 (69.2)		2.6 (4.6)	0 (−0.2)	0.3 (7.7)	
4	26.9 (49.5)	31.6 (40.9)	14.3 (21.8)	2.9 (15.0)		
5	31.4 (71.9)	7.5 (7.5)	6.8 (8.4)	0 (−9.4)		
6	16.5 (19.0)	15.9 (15.9)	2.0 (2.6)	3.6 (10.0)		
7	7.0 (13.7)					
8	8.0 (35.4)					
9	14.8 (33.8)					
10	23.2 (30.9)					
11	22.3 (59.8)					
12	27.3 (59.6)					
13	7.9 (32.8)				1.5 (2.8)	
14	8.5 (14.4)				0 (1.2)	
15	15.3 (30.4)				17.9 (20.1)	
16	26.0 (27.3)					
17	20.4 (29.0)					
18	26.9 (31.6)					
19	TF	1.0 (1.2)	2.5 (2.5)	6.9 (9.8)		
20	22.9 (29.6)	0 (0.2)	28.7 (30.8)	2.2 (2.2)		
21	22.5 (28.4)	2.7 (3.6)	13.6 (13.6)			
22	11.6 (19.6)			0 (0.5)		
23	10.4 (21.3)			2.7 (4.5)		
24	9.6 (16.4)			1.7 (1.7)		

Pretreatment: 250 IU IFN-α, ml^{-1} for 18 h; TF, technical fault
Results indicate % CMC by IFN treated − % CMC by untreated lymphocytes
[a] Figures in parentheses are % CMC values after IFN
[b] E : T ratio 20 : 1; against all other targets, 40 : 1

with 10 with IFN pretreatment. Out of the total of 116 effector : leukaemia cell combinations tested, one was positive without IFN, as against 22 that were positive with IFN. Seven leukaemias were susceptible to IAK by more than one donor and included one AML, three AMML, two ALL, and one immunoblastic lymphoma. There was no correlation between susceptibility to IAK, the morphological type of leukaemia, and the induction of remission in those patients.

Table 4. Summary of IAK on fresh leukaemias

Cell code	Diagnosis[a]	Remission	CMC tests positive/total tests after		Susceptible to IAK[d]
			No treatment	Interferon	
E69	AML	−	0/3	0/3	−
E70	AMML	−	0/6	0/6	−
E71	AML	−	0/6	0/6	−
E72	AML	−	0/6	1/6	±
E73	AMML	+	0/3	0/3	−
E75	AML	−	0/9	0/9	−
E76	AML	−	0/6	4/6	++
E77	ALL	+	0/6	2/6	+
E78	ALL	+	0/3	0/3	−
E79	ALL	+	0/9	3/9	++
E81	ALL	+	1/11	1/11	±
E82	AML	+	0/3	0/3	−
E83	CML→ALL	+	0/3	0/3	−
E84	AMML	+	0/6	5/6	++
E85	AMML	+	0/6	1/6	±
E86	Lymph		1/3	2/3	+
E87	AMML	?+	0/6	2/6	+
E88	AML	+	0/3	0/3	−
E89	ALL	−	0/3	1/3	±
E90	AML	+	0/6	0/6	−
E91	ALL	+	0/6	0/6	−
EJU	Ph^+Bl.Cr, CML	−	0/3	0/3	−
K562	E−AML−CML		23/23	23/23	+++
Total: Cells tested:					
	AML: 8				2
	AMML: 5				3
	ALL[b]: 9				5

[a] See Table 1 for further details
[b] Includes E83, E86, EJU
[c] Positive CMC > 10%
[d] +, IAK-susceptible; −, resistant; ±, weakly susceptible; +, ++, +++, increasing degrees of susceptibility

Discussion

It was clear from this study that the sensitivity of acute leukaemias to CMC rarely approached that of the cell line K562, either with or without IFN pretreatment of the effector population. The ability of IFN to amplify donor lymphocyte NK-CMC was assessed on K562 target cells, as previously described [19], and though higher effector concentrations of treated lymphocytes were tested they generally gave CMC values lower than 10% on fresh leukaemias. In comparison with CMC on K562, fresh leukaemias are thus largely resistant to NK, which is in keeping with the results of other studies [23, 27, 38]. Amplification of NK-CMC by IFN resulted in more consistent lysis of acute leukaemias, though only a minority ($n = 10$) of those tested ($n = 22$) with a proportion of donors were susceptible. Of the 12 leukaemias resistant to IAK, seven were tested with only three donors, and four with six donors (Table 4). Only one leukaemia (E75) was resistant when tested against more than six donors. This suggests that each leukaemia tested with a larger number of donors might have revealed positive CMC. Thus, susceptibility of leukaemias to lysis by IAK involves consideration of the lytic potential of the effector cell, together with the donor-relatedness of IAK on each leukaemia. It is unlikely that leukaemias are intrinsically refractory to CMC [15, 31, 37], suggesting that lymphocytes with antileukaemic reactivity may be present in certain donors at levels too low for detection in most preparations of normal leucocytes. Although several leukaemias were resistant even at very high E : T ratios the recent availability of procedures for the enrichment of NK cells [32] renders this question amenable to further examination.

The induction of target cell resistance was avoided in the experiments by pre-incubating effectors with IFN and thereby eliminating any protective IFN-target cell interaction [21, 33]. However, other factors may be involved in resistance relating, for example, to the stage of differentiation of the target cells [4] and/or to their capacity for effective membrane repair [14].

Although every donor lymphocyte preparation treated with IFN was, without exception, capable of amplified CMC against K562 cells, the increment of amplified CMC was not predictive of any antileukaemic effect. This observation implies some degree of heterogeneity with respect to the determinants expressed by different targets and/or receptors on the effector cells. It is feasible that within the same general subpopulation there are functionally distinct populations of cytotoxic cells, varying in number between different subjects, in their response to interferon, and in the expression of NK receptors for specificities on different targets. There are several lines of evidence which support this, one example of which is the variable effect of cold inhibition by one NK-sensitive target on NK-induced lysis of another such target [see review by Herberman et al. 7]. It is relevant here that a recent successful attempt to clone mouse NK cells revealed that antigen receptors on NK cells are not clonally distributed, but left open the possibilities that NK cells either recognize

common structures on NK-sensitive targets, or carry many receptors each with different specificity [3].

Our data corroborate and extend those of Zarling et al. [38], who also found that anti-K562 activity could not predict for leukaemia sensitivity and that disclosure of susceptibility required simultaneous testing with several donors. In this and other human tumour systems [34], IFN-induced enhancement of allogeneic killing, in the absence of comparable reactivity by autologous lymphocytes, has been interpreted on the basis of alloantigenic recognition and a lytic effect due to IFN activation [13]. According to this, fresh tumour cells of whatever provenance appear not to share the membrane properties of the in vitro adapted cell lines, which exhibit high sensitivity to natural and activated killer cells without the involvement of antigen recognition.

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