ROLE OF ALLOANTIGENS IN NATURAL KILLING

Allogeneic but Not Autologous Tumor Biopsy Cells Are Sensitive for Interferon-induced Cytotoxicity of Human Blood Lymphocytes*

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Interferon (IF)[1] is known for its effect on virus cell interactions (1). The first indication that it could influence cellular cytotoxicity was provided by Svet-Moldavsky and Chernyakovskaya in 1967 (2), when they observed that lymphocyte-mediated killing was enhanced in the presence of IF. Their system comprised mouse lymphocytes and the L cell line. This finding was not followed up until 1972 when Lindahl et al. (3) reported that IF enhanced the specific cytotoxicity of allosensitized murine T cells. Recent experiments established that exposure of lymphocytes to IF in vitro elevates their natural killer (NK) activity (4–6). In addition, treatment of the lymphocyte donors with IF or IF inducers also leads to increased in vitro cytotoxic potential (7–9). The cytotoxic system is influenced in the opposite direction if the targets are treated with IF inasmuch as their susceptibility for killing is decreased (10).

IF is used in tumor therapy because of its antitumor (11), antiviral, and immunopotentiating effects. Beneficial effects have been reported for Hodgkin's lymphoma (12), myelomatosis (13), malignant lymphoma, (14) and leukemia (15).

In the majority of studies dealing with NK and the IF-activated killing (IAK), established cell lines were used as targets. In experimental systems, freshly harvested cells usually had lower NK sensitivity than cultured lines (16, 17). Also, in man, tumor cells isolated from biopsies were generally NK resistent, i.e., they were not killed by allogeneic lymphocytes derived from healthy donors (18). On the other hand, a tumor-specific reactivity was indicated in 28% of 156 cases because the biopsy cells were damaged by the autologous lymphocytes (19). We refer to this effect as autologous lymphocyte-mediated cytotoxicity (ALC).

The experiments reported in this paper were initiated with the aim to investigate whether IF treatment in vitro influences the ALC. Such effects would have provided an in vitro correlate for the therapeutical effect of IF. The results were negative inasmuch as no ALC was induced. The ALC-negative cases remained negative, and no elevation of the existing effects occurred.

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[1] *Abbreviations used in this paper:* ALC, autologous lymphocyte-mediated cytotoxicity; ATS, autologous tumor stimulation; Con A, concanavalin A; FI, Ficoll-Isopaque; IAK, interferon-activated killing; IF, interferon; MHC, major histocompatibility complex; NHS, normal human sera(um); NK, natural killer.

An interesting phenomenon, however, was discovered in that IF-induced cytotoxicity was efficient only in allogeneic combinations. Because the IAK has been shown to be performed by those lymphocyte subsets that are also responsible for the NK (20, 21), this finding may contribute to the understanding of the NK mechanism. It is likely that IF activates the cytotoxic potential of the lymphocyte population, and that those cells that carry the receptors for the histocompatibility antigens expressed on the particular target can manifest the cytotoxic function.

Materials and Methods

Patients. 43 patients with solid tumors of various histologic types were studied (Table I). Except for eight patients (1019, 1020, 1023, 1024, 1027, 1032, 1034, and 1035) who had osteosarcomas and were receiving IF (3 million U/d) and seven patients (1127, 1120, 1113, 0788, 0508, 0509, 0510) who were operated earlier for their primary tumors, the patients were not treated before the tumor and blood sampling.

Tumor Cells. These cells were separated from biopsy specimens according to our procedure described elsewhere (22), by the stepwise application of velocity and density sedimentations on Ficoll-Isopaque (FI) (Pharmacia Fine Chemicals, Inc., Upsala, Sweden) and/or human serum albumin gradients, treatment with collagenase and DNase, and adherence to plastic surface. The isolated tumor cells were incubated overnight in culture conditions before the cytotoxic test to allow regeneration of putative antigens assumed to be affected during the procedures of cell separation. Experiments were performed only with cell suspensions that had at least 80% cell viability, as assessed by trypan blue exclusion, and had <5% obvious contamination with nonmalignant cells.

Storage of Tumor Cells. Samples of tumor cells ($2-5 \times 10^6$) were stored in liquid nitrogen for use as targets in repeated tests. The cells were suspended in 0.5 ml of RPMI-1640 medium (Flow Laboratories, Ltd., Irvine, Ayrshire, Scotland) that contained 40% normal human serum (NHS), after which 0.5 ml of 20% dimethyl sulfoxide in RPMI-1640 medium was added dropwise. Ampules were frozen at 1°C/min. After thawing, cells were diluted to 10 ml with RPMI-1640 medium plus 20% NHS, centrifuged, and resuspended in RPMI-1640 medium plus 10% NHS. When necessary, dead cells were removed by centrifugation on FI gradients.

Cell Lines. The erythroid line K-562 was derived from a patient with chronic myeloid leukemia in blast crisis (23). The T cell line Molt-4 was obtained from a patient with acute lymphoid leukemia (24). They were maintained in suspension culture in RPMI-1640 medium supplemented with 10% fetal calf serum.

Lymphocytes. Lymphocytes from 43 patients and 49 healthy donors were separated from heparinized blood on FI, followed by incubation in Falcon plastic culture flasks (type 3024, Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.) for 30 min at 37°C and by passage through a nylon fiber column (25). The nylon fiber column-passed populations contained 91 ± 6% sheep erythrocyte-rosetting cells. Contamination with cells of obviously nonlymphocyte morphology represented <1%.

IF Preparations. The partially purified Sendai virus-induced human leukocyte IF preparations were received from Dr. Kari Cantell, Central Public Health Laboratory, Helsinki, Finland. They were produced and purified as previously described (26). The antiviral activities of the preparations were determined by assaying inhibition of plaques induced by vesicular stomatitis virus in human amnion (U) cells (27) and expressed in international reference units by comparison with the international reference preparation 69/19. The antiviral activities of the preparations were $\sim 6 \times 10^6$ U/ml, with an $\sim 10^6$ U/mg of protein sp act.

IF Treatment of the Lymphocytes. Aliquots of lymphocytes ($3-10 \times 10^6$) were incubated with or without 1,000 U/ml IF for 1-3 h at 37°C in culture medium. The cells were then washed and used as effectors in the cytotoxicity tests. IF treatment of the lymphocytes had no effect on cell viability as assessed after crystal violet staining. The cytotoxicity of these lymphocytes will be referred to as IAK.

Medium. RPMI-1640 medium with L-glutamine (200 mM solution, 1% by vol), benzylpenicillin (100 IU/ml), streptomycin sulfate (100 μg/ml), Hepes buffer (0.1 mM/ml), and heat-

TABLE I
List of Patients

Patient number	Sex	Age	Diagnosis	Clinical stage	Differentiation*
		yr			
1019	M	20	Osteosarcoma	Localized	L
1020	M	20	Osteosarcoma	Localized	L
1023	M	15	Osteosarcoma	Lung metastasis	L
1024	F	14	Osteosarcoma	Localized	L
1027	M	25	Osteosarcoma	Localized	L
1032	M	15	Osteosarcoma	Localized	L
1034	M	17	Osteosarcoma	Localized	L
1035	M	11	Osteosarcoma	Lung metastasis	L
1025	M	51	Malignant chordoma	Local recurrence	D
1028	M	74	Malignant mesenchymal tumor	Lung metastasis	U
1031	M	70	Malignant mesenchymal tumor	Localized	U
1115	F	77	Malignant mesenchymal tumor	Localized	U
1131	M	75	Malignant histiocytic tumor	Lymph node metastasis	L
1033	M	43	Fibrosarcoma of the tibia	Localized	L
1126	M	59	Fibrosarcoma	Localized	L
1130	F	71	Myxoid fibrosarcoma	Localized	L
1127	M	77	Liposarcoma	Local recurrence	L
0111	M	43	Liposarcoma	Localized	L
1120	M	52	Myxoid liposarcoma	Local recurrence	D
1121	F	60	Myxoid liposarcoma	Localized	D
1117	F	76	Synovial sarcoma	Localized	L
1113	F	60	Melanoma	Lung metastasis	D
0788	F	46	Melanoma	Brain metastasis	D
2256	F	52	Thymoma	Localized	D
2258	M	59	Thymoma	Localized	D
2259	F	58	Thymoma	Localized	D
0500	M	55	Hypernephroma	Localized	D
0508	M	54	Hypernephroma	Lung metastasis	L
0509	M	66	Hypernephroma	Lung metastasis	L
0510	F	70	Hypernephroma	Brain metastasis	L
2217	M	69	Adenocarcinoma of the lung	Lymph node metastasis	D
2224	M	62	Adenocarcinoma of the lung	Localized	L
2229	F	51	Adenocarcinoma of the lung	Localized	D
0470	M	80	Adenocarcinoma of the lung	Localized	D
2225	M	63	Squamous cell carcinoma of the lung	Localized	L
2234	F	59	Squamous cell carcinoma of the lung	Localized	D
2244	M	51	Squamous cell carcinoma of the lung	Localized	L
2255	M	50	Squamous cell carcinoma of the lung	Localized	L
0012	M	72	Squamous cell carcinoma of the lung	Inoperable	L
0013	M	61	Squamous cell carcinoma of the lung	Inoperable	D
2219	M	67	Oat cell carcinoma of the lung	Lymph node metastasis	U
0784	F	51	Astrocytoma grade III		
0787	F	25	Astrocytoma grade II		

* L, low differentiation; U, undifferentiated; D, differentiated.

inactivated serum (from healthy male donors) added in 10% concentration was used in all experiments. NHS were not preselected, but cloudy sera were excluded.

Cytotoxicity Assay. A modification of the method described by Vose et al. (28) has been used. Target cells (10^6) were labeled in 0.5 ml medium by the addition of 100 μCi ^{51}Cr (as sodium

TABLE II

Effect of IF on the Cytotoxicity of Lymphocytes Against Autologous and Allogeneic Tumor Biopsy Cells and Against K-562

Lymphocytes			Targets		
Donor		Incubated with IF*	Tumor biopsy cell number		K-562
Number	Diagnosis		1115	2219	
1115	Malignant mesenchymal tumor	−	8 (27)‡	5 (37)	22 (25)
		+	6	44§	31
0111	Liposarcoma	−	22	4	19
		+	76‖	27¶	41§
	Healthy	−	11	4	25
		+	33§	22¶	38§

* The lymphocytes were incubated with 1,000 U/ml IF for 1 h and washed before the test.

‡ The values represent the percent specific ^{51}Cr release and those in parentheses indicate the percent spontaneous ^{51}Cr release.

§ $P < 0.01$ between the values obtained with lymphocytes with and without IF treatment.

‖ $P < 0.001$ between the values obtained with lymphocytes with and without IF treatment.

¶ $P < 0.05$ between the values obtained with lymphocytes with and without IF treatment.

chromate; 100–350 μCi/μg sp act; The Radiochemical Centre, Amersham, Buckinghamshire, England). After incubation for 2 h at 37°C the cells were washed twice. They were further incubated for 1 h, washed twice, and resuspended in culture medium. Target cells (5×10^3/well) were dispensed into wells of round-bottom microplates (96 wells/plate; Titertek, Flow Laboratories, Ltd.). Lymphocytes were then added to give an effector:target ratio of 50:1. The final vol in each well was 0.2 ml. The plates were centrifuged for 10 s at 800 *g*. After a 4-h incubation at 37°C, the supernates and the pellets were collected separately (by means of Titertek) and the radioactivity in the supernate and pellet was measured in a gamma counter. Spontaneous ^{51}Cr release was measured from target cells incubated in medium; maximum ^{51}Cr release was obtained by lysis of the cells with detergent (Triton X-100, Sigma Chemical Co., St. Louis, Mo.).

Test evaluation. The percentage of ^{51}Cr release was calculated from the following formula: (counts in supernate/counts in supernate and pellet) × 100. The percent specific ^{51}Cr release was then determined by the following formula: (percent release in test − percent spontaneous release)/(percent maximum release − percent spontaneous release) × 100. Tests in which the spontaneous release exceeded 50% were disregarded. Statistical significances were calculated on the triplicates by the Student's *t* test. A further criteria for the positivity of ALC was that the level of specific ^{51}Cr release had to be twice over the control. This was obtained by the experiments performed between lymphocytes from 47 healthy donors and tumor biopsy cells from 45 patients. On the basis of these results 20% specific release was chosen as limit for positivity. Specific ^{51}Cr release >20% was always significant by the Mann-Whitney U test and was arbitrarily chosen as limit for positivity. This may be too high, and, therefore, it is likely that some of the positive effects are not considered. In the present series, the cytotoxic efficiencies of untreated and IF-treated lymphocytes were compared; thus, the limit for determining the positivity is of secondary importance. Positivity for changes of cytotoxicity caused by IF was established on the basis of statistical significance between counts per minute values of the triplicates.

Results

Effect of IF on Lymphocyte Cytotoxicity Against Primary Tumor Biopsy Cells. Table II presents the results of an experiment in which two patients (malignant mesenchymal tumor 1115 and liposarcoma 0111) and one healthy donor were involved. After pretreatment of the lymphocytes with IF, the NK activities were measured as the

TABLE III

Effect of IF on the Cytotoxicity of Lymphocytes Against Autologous and Allogeneic Tumor Biopsy Cells and Against Molt-4

Lymphocytes			Targets		
Donor		Incubated with IF*	Tumor biopsy cell number		Molt-4
Number	Diagnosis		0509	0508	
0509	Hypernephroma	−	50 (42)‡	10 (38)	14 (24)
0509	Hypernephroma	+	45	26§	21
0508	Hypernephroma	−	2	0	32
0508	Hypernephroma	+	47‖	5	40§
	Healthy	−	43	24	89
	Healthy	+	62‖	83‖	83

* The lymphocytes were incubated with 1,000 U/ml IF for 1 h and washed before the test.

‡ The values represent the percent specific ^{51}Cr release and those in parentheses indicate the percent spontaneous ^{51}Cr release.

§ $P < 0.05$ between the values obtained with lymphocytes with and without IF treatment.

‖ $P < 0.001$ between the values obtained with lymphocytes with and without IF treatment.

elevated anti-K-562 effect. Except for the effect of the 0111 lymphocytes on the 1115 targets, the effects against primary biopsy cells were weaker than against K-562. IF treatment regularly enhanced the killing of allogeneic targets. This was not the case for ALC.

The results of an experiment with lymphocytes from two hypernephroma patients and one healthy blood donor are given in Table III. The NK activity of the patients, as measured against Molt-4, was weaker than that of the control donor. The strong NK activity of the lymphocytes of the healthy donor is also reflected by the cytotoxicity against the biopsies. The lymphocytes of patient 0509 killed the autologous tumor cells. This activity was not elevated by IF pretreatment, whereas allo-killing was induced. The lymphocytes of patient 0508 were inactive against both the autologous and the allogeneic tumor cells. IF treatment of the lymphocytes induced strong cytotoxicity against the allogeneic biopsy cells.

Table IV shows a test with lymphocytes and tumor cells from a synovial sarcoma (1117) and an osteosarcoma (1020) patient. The standard NK activity (anti-K-562) of the lymphocytes of the patients was autgmented by IF. Cytotoxicity against the biopsy cells was increased by IF only in the allogeneic combinations.

The summary of ALC experiments with lymphocytes from 25 tumor patients is presented in Fig. 1 a and Table V. ALC occurred in 7 (28%) cases. In an additional four cases, the specific ^{51}Cr release was between 10 and 20%. In 24 of 25 cases, incubation of the lymphocytes with IF did not significantly change the level of ALC, whereas in one it decreased the killing potential (of the 24 cases in which the change was not significant, the ^{51}Cr-release values were higher in 10 and lower in 10 cases).

The results of cytotoxicity against allogeneic biopsy targets with lymphocytes from 46 healthy donors and 24 tumor patients are presented in Fig. 1 b and c, respectively. The controls were active in 7 of 50 (14%) tests (Fig. 1 b), and, in 5 of these 7, IF increased the cytotoxic efficiency. Of the 43 negative tests, IF treatment caused significant cytotoxicity in 22. The specific ^{51}Cr release was somewhat enhanced in an additional 10 cases, diminished in 7, and equal to that of untreated samples in 6. The

TABLE IV

Effect of IF on the Cytotoxicity of Lymphocytes Against Autologous and Allogeneic Tumor Biopsy Cells and Against K-562

Lymphocytes			Targets		
Donor		Incubated with IF*	Tumor biopsy cell number		K-562
Number	Diagnosis		1117	1020	
1117	Synovial sarcoma	−	17 (40)‡	4 (44)	36 (28)
1117	Synovial sarcoma	+	20	14	57§
1020	Osteosarcoma	−	9	20	4
1020	Osteosarcoma	+	31‖	22	26§
	Healthy	−	7	0	28
	Healthy	+	30‖	33‖	32

* The lymphocytes were incubated with 1,000 U/ml IF for 3 h and washed before the test.

‡ The values represent the percent specific ^{51}Cr release and those in parentheses indicate the percent spontaneous ^{51}Cr release.

§ $P < 0.01$ between the values obtained with lymphocytes with and without IF treatment.

‖ $P < 0.001$ between the values obtained with lymphocytes with and without IF treatment.

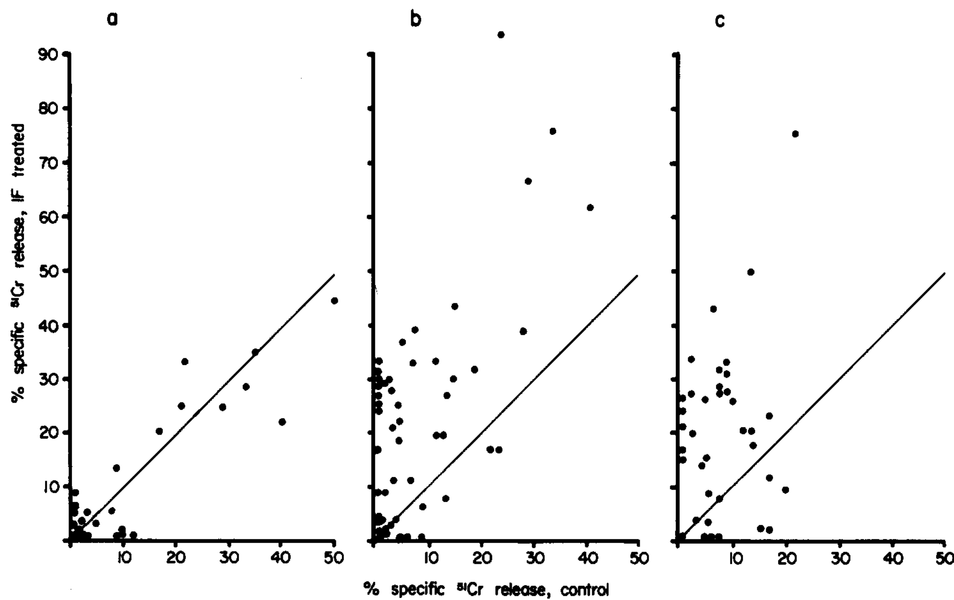

FIG. 1. Influence of IF on the cytotoxic potential of lymphocytes exerted on primary tumor cells (a) in autologous combination, (b) by lymphocytes of healthy donors, and (c) by lymphocytes of tumor patients towards allogeneic target cells.

increase (± SE) in the mean percent specific ^{51}Cr release with IF-treated lymphocytes from healthy donors was 12 ± 2.6, and this was statistically significant ($P < 0.001$).

The results with the tumor patients were similar. They showed a significantly lower proportion of allogeneic cytotoxicity, i.e., in 2 of 37 (5%) of the tests (Fig. 1 c). In 18 of 37 tests, IF-treated lymphocytes exhibited significant cytotoxicity. The specific ^{51}Cr release was elevated in an additional eight cases, diminished in eight, and equal to

TABLE V

IF-induced Cytotoxicity of Blood Lymphocytes Against Tumor Biopsy Cells

Targets (tumor biopsy cells)	Effector lymphocyte from					
	Tumor patient			Healthy donor		
	IF−	IF+	Change in percent ^{51}Cr release	IF−	IF+	Change in percent ^{51}Cr release
Autologous	7/25 (28%)*	7/25 (28%)	−0.3 ± 1.6‡	—	—	
Allogeneic	2/37 (5%)	18/37 (49%)	12.7 ± 2.6	7/50 (14%)	26/50 (52%)	14.9 ± 2.2

* Cases with statistically significant cytotoxicity per number of tests.

‡ Mean percent increase of the specific ^{51}Cr release ± SE, as a result of IF treatment of the effectors.

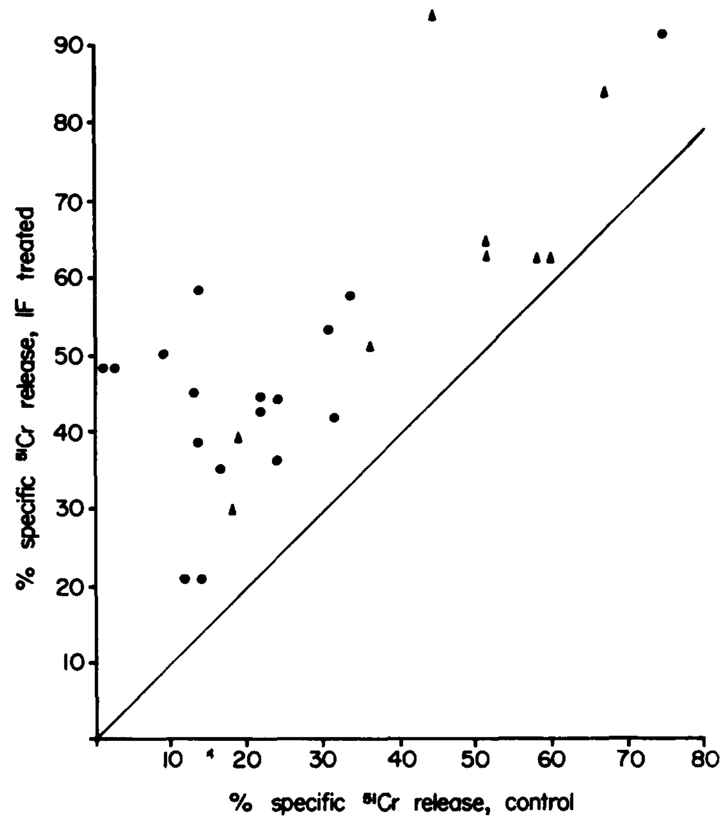

FIG. 2. Influence of IF on the (▲) anti-K-562 and (●) anti-Molt-4 activity of lymphocytes of tumor patients.

that of untreated lymphocytes in three. The difference in the mean percentage specific ^{51}Cr release (± SE) between the IAK and NK was 14.9 ± 2.2, and this was also statistically significant ($P < 0.001$).

A summary of the IAK results is given in Table V. ALC was similar with IF-treated and untreated lymphocytes. The mean percentage of difference was −0.3 ± 1.6. The

TABLE VI

Elevated Susceptibility of Cultured Tumor Biopsy Cells for the Cytotoxic Effect of Autologous and Allogeneic Lymphocytes

Lymphocyte donor number	Incubation with IF*	Tumor donor number	Percent specific cytotoxicity of target cells		
			Cultured‡	Kept frozen	Used fresh
2259	−	2259	21 (22)§	8 (27)	3 (31)
2259	+	2259	26	13	0
Healthy donor	−	2259	36	15	NT
Healthy donor	+	2259	68 ‖	43¶	NT
0788	−	0788	22 (26)	4 (32)	0 (38)
0788	+	0788	37**	2	2
Healthy donor	−	0788	20	4	NT
Healthy donor	+	0788	25	13	NT
0510	−	0510	5 (37)	2 (29)	0 (34)
0510	+	0510	34¶	4	3
1131	−	0510	2	0	NT
1131	+	0510	27 ‖	24¶	NT
Healthy donor	−	0510	0	NT	5
Healthy donor	+	0510	20 ‖	NT	0

* The lymphocytes were incubated with 1,000 U/ml IF for 1 h and washed before the test.
‡ The target cells were cultured for 5 d in RPMI-1640 medium enriched with 10% NHS.
§ The values represent the percent specific ^{51}Cr release and those in parentheses indicate the percent spontaneous ^{51}Cr release. NT, not tested.
‖ See Table II.
¶ See Table II.
** $P < 0.01$ between the values obtained with lymphocytes with and without IF treatment.

lower NK efficiency of the lymphocytes of the tumor patients is reflected by the lower frequency of allo-killing (5%) compared with the 14% obtained with effectors from healthy donors. There was no significant difference between the proportion of IAK-positive tests of the patients and controls with allogeneic biopsy targets (49 and 52%, respectively).

Eight osteosarcoma patients received IF therapy at the time of tumor and blood sampling. The results with this group of patients did not differ from the general pattern either in the efficiency of the effectors or the sensitivity of the targets.

Lymphocytes from 12 patients were assayed against the K-562 and/or Molt-4 lines. In all 26 tests (Fig. 2) the IAK was elevated over the NK. Repeated tests gave similar results. In accordance with previous findings, the NK efficiency of lymphocytes from healthy donors was stronger than that of the tumor patients. The mean ^{51}Cr-release values in healthy donors and tumor patients was 44.8 and 14.5% for K-562, and for Molt-4 63.4 and 27.6%, respectively.

Alteration of the Cytotoxic Susceptibility of Biopsy Cells after Cultivation. In three experiments, the tumor cells were used as targets directly, after 5–6 d of cultivation, or after preservation in frozen state. The effector cells were used with and without pretreatment with IF (Table VI). In accordance with the above described results, IF did not affect the reactivity against fresh or frozen stored autologous cells. Lymphocytes of three healthy individuals and of one tumor patient (1131) showed no NK against fresh or frozen stored allogeneic targets, whereas IAK was efficient in two of four

tests. After 6 d in culture, the cytotoxic sensitivity of the biopsy cells increased, and, in two of three tests, the ALC was positive (2259 and 0788). All three tests were positive after IF treatment of the lymphocytes.

In two of four cases, the cultured targets were NK sensitive, and in all four tests IAK sensitive.

Discussion

The lack of induction of ALC by in vitro IF treatment of the lymphocytes does not exclude the fact that in the complexity of the prevailing events, in vivo administration of IF can enhance the antitumor activity of the immune system. The immune response comprises interactions between several components, humoral and cellular, and the outcome might be influenced by IF acting on other levels than the direct cytotoxic effect of lymphocytes.

In previous experiments, ALC, i.e., cytotoxicity of the patients lymphocytes against their own tumor cells, was obtained in 27% of 198 cases. The effects were usually weak. The reactivity was exerted by the T cell-enriched subset and mainly autologous tumor cells were affected (29, 18). Although not proven, we may consider the in vitro cytotoxicity as a manifestation of antitumor autoimmunity. Induction of DNA synthesis in lymphocytes by the autologous tumor biopsy cells (autologous tumor stimulation [ATS]) is an additional parameter that suggests the occurrance of a tumor-specific cellular reaction (30, 31). ATS was demonstrated in higher proportion of cases (68%) than ALC (32). The reason why ALC was not influenced by IF treatment of the lymphocytes is unclear. It may be a result of the low number of antigen-recognizing lymphocytes and/or the weak expression of the relevant antigens on the targets.

In studies concerned with the characteristics of the NK, freshly harvested targets were usually resistent or were weakly sensitive in short-term cytotoxicity tests (16). In accordance, human tumor biopsy cells were rarely killed by allogeneic lymphocytes (18, 19). As shown in our results, when killing occurred, the effectors were exceptionally active against the standard NK targets.

In one study, designed to clarify the conditions that influence the NK susceptibility of the target, it was shown that the sensitivity of the same mouse tumor line changed depending upon whether it was derived from cultures or from animals (17). Our results with the human biopsy cells show a similar phenomenon. The reactivities that appear after in vitro cultivation may be the consequence to a quantitative or qualitative change in the expression of surface antigens. Such changes after explantation have in fact been demonstrated in a murine lymphoma system both for the histocompatibility (decrease) and virus-determined antigens (increase) (33). The TL antigen expressed on certain murine leukemias was also found to be modulated, it was detectable on cells kept in vitro although absent from cells harvested from immunized animals (34). Another alternative may be that in the conditions prevailing in culture those cell membrane properties that determine the sensitivity for cytotoxicity are changed. An increase in the sensitivity toward complement-mediated immune killing was observed after treatment of the targets with antimetabolites, perhaps because of an effect on cell membrane repair mechanisms (35).

We may also consider the possibility that IF is responsible for the relatively low sensitivity of primary cells (10). Immune IF may be produced in the patients in the

course of an anti-tumor response, and this may cause the relatively low NK sensitivity of the tumor cells. These alternatives are speculative, and it remains to be seen what the factor is that is responsible for the changed susceptibility toward lymphocyte-mediated killing after explantation.

The experiments revealed an interesting aspect concerning the involvement of the histocompatibility antigens in the natural killing. In the majority of NK studies, cultured lines and most often tumor lines were used as targets. The recognition seems not to be specific because sensitive cells are killed without discrimination. Tests performed with lymphocytes, of various individuals often differ in efficiency, but the ranking order of damage exerted on the various targets is similar (36, 37).

NK phenomenon is operationally defined as the cytotoxicity exerted by lymphocytes harvested from individuals without known sensitization history toward the target. IF treatment is known to enhance this effect, and the effector cells of NK and IAK were shown to be in the same subsets (20).[2] IAK performed with the lymphocytes of donors without known immunization toward the target may be regarded as an enhanced NK phenomenon and also affects such targets that have low NK sensitivity. We may consider the situation as follows: The individual lymphocytes in the population function at various levels of intensity. For the different targets, the threshold of the intensity necessary to bring about damage seems to differ, hence the differences in the proportion of target killed by a given number of effector cells. It is unknown whether the critical factor acts at the event of establishment of the contact or at the event of the lethal hit. The results of cold target inhibition experiments would suggest the former because the competitive capacity of a particular target usually parallels its cytotoxic sensitivity. Two sets of experiments provided evidence that the elevated IAK, as compared with NK, in a given system is a result of recruitment of new cells that can kill the target, rather than action on the already active cells in such a way that each kill an increased number of targets. These experiments were (*a*) comparative limiting dilutions of effectors in NK and IAK using the same target, which showed an increased number of active samples in IAK;[2] and (*b*) elimination of NK cells by preexposure of the lymphocytes to fibroblast monolayer that left potentially active cells inducible by IF treatment (38). In addition, results with an in vivo system also pointed to this mechanism. When rats were depleted of NK activity by radiation or drug treatment and injected with IF inducers, NK cells were found to reappear within a short time (39).

A likely explanation for the allogeneic killing induced by IF in our experiments is a polyclonal activation of the cytotoxic potential that is then manifested by the lymphocytes committed to the alloantigen specificities expressed on the particular target cells. The role of the specific receptors may be the establishment of contact between the interacting cells. Experiments concerned with the nature of NK and IAK have not yet revealed the recognition of alloantigens, although it may be the cause of a certain degree of specificity and is often detectable superimposed on cross-reactivities. Some indications for the recognition of alloantigens were seen when in vivo activated murine killer cells were tested on macrophage targets in a 16-h assay. The effect was weaker on syngeneic compared with allogeneic targets (40). However, in other series

[2] Masucci, M. G., G. Masucci, E. Klein, and W. Berthold. Interferon induced cytotoxicity of human lymphocytes. Manuscript submitted for publication.

of experiments efficient killing of syngeneic primary target of nontumor origin such as thymus cells was reported (41). These latter effects may, however, also represent a killing as a result of the recognition of cell surface antigens. Mice are known to harbor endogenous viruses that impose surface antigens on certain cell types, and these are known to be recognized by the immune system (42). It seems that the conditions to reveal the alloantigen-determined killing have to be strict in the sense that cultured target cells have to be avoided.

The activation of cytotoxic precursors by IF and the manifestation of cytotoxicity toward cells that carry alien histocompatibility antigens is similar to the findings with mitogen-activated murine cells. The murine system has the advantage that the influence of the major histocompatibility antigens can be easily studied in detail with co-isogeneic strains. The phenomenon described by several authors (43–45) is as follows: Spleen cells were activated in vitro with Concanavalin A (Con A), and this killing effect was tested after the lectin was removed by incubation with α-methyl-D-mannoside. Cytotoxicity was exerted by the activated lymphocytes only toward targets that expressed alien major histocompatibility complex (MHC) locus-determined antigens. Differences in the loci of minor histocompatibility antigens and expression of tumor-related antigens were not sufficient to bring about this reaction. This type of cytotoxicity differed from that obtained in the presence of lectin, which was indiscriminative and also affected histocompatibile cells. The interpretation proposed was that Con A activates precursors for expression of cytotoxic potential and in a particular test the clone that was committed to the MHC antigens present on the target exerted the killing. Because it is known that the frequency of lymphocytes that recognize MHC antigens is high, such cytotoxicities are often measurable without the necessity of the enlargement of the reactive clone. This mechanism may also be applicable to the NK and IAK systems due to the restriction to allogeneic targets.

The search for a particular antigen responsible for the NK effect did not give support for the existence of a well-defined entity. It is possible that membrane properties of cultured cell lines contribute to the interaction with the killer cells. Similar to the cytotoxicity in the presence of lectins, such interactions have a certain specificity inasmuch as targets from the same species are affected with considerably higher efficiency than xenogeneic targets that are sensitive to the NK exerted by the lymphocytes of their own species (36, 46). It is known that the number of lymphocytes that recognize cell surface antigens of an alien species is relatively low. A species-specific recognition on the cell membrane level has also been indicated when T cells and thymocytes were admixed to various target cells. They attached to cells in a species-specific pattern, distinguishing their own and closely related species (47).

The cell membrane property acquired in culture conditions that contribute to the suicidal interaction with the lymphocytes is unknown. With freshly harvested cells this factor is absent, and the effect exhibited by lymphocytes of the unprimed donors (NK) may be the consequence of a certain, individually variable, degree of T cell activation (the active cells have been shown to be within the T subset) (48, 49). Those activated T cells that recognize the antigens determined by MHC on the target can establish the contact and exert the killing function. IF or IF inducers recruit potential killer cells. All evidences point to the fact that the lymphocyte cytotoxicities without manipulation and after IF activation are similar in the ranking order of the efficiencies exhibited by various subsets and in the sensitivities of various targets. According to

this view, the NK (and IAK) effect would be related to cytotoxic T lymphocytes when assayed on fresh targets, whereas with some cultured cell lines tested in short-term assays, as a result of yet undefined properties of their plasma membrane, events similar to the cytotoxicity in the presence of lectins could occur.

Summary

Blood lymphocytes of patients with solid tumors were assayed for cytotoxicity against autologous and allogeneic primary tumor cells. The lymphocytes killed autologous tumor cells in 7 of 25 cases (28%) and allogeneic tumor cells in 2 of 37 tests (5%). Lymphocytes from healthy donors were rarely cytotoxic for the biopsy cells, which indicates that these cells have low natural killer sensitivity.

The autoreactivity that may reflect the immunological recognition of tumor cells was not altered by pretreatment of the effectors with interferon (IF). In contrast, killing of allogeneic tumor biopsy cells was induced by IF in ~50% of tests, with the lymphocytes of both the tumor patients and the healthy donors. The mechanism of the alloreactivity is most likely a consequence of IF-induced polyclonal activation of cytotoxic potential and the lymphocytes that are committed to recognize the alloantigens expressed on the particular target manifest the killing function.

When the biopsy cells were explanted and kept in culture for 5-6 d, their susceptibility for the lymphocyte damage increased, and they were killed by the IF-treated cells also in autologous combinations. Whether this change in sensitivity is a result of qualitative or quantitative changes in antigen expression or of other changes in the properties of the cell membrane is unknown.

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