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Interferon effect on cytolytic T lymphocytes in a single cycle assay

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Summary. In this study we analyse cell-mediated cytotoxicity by the use of ^{51}chromium release and the killer cell enumeration assay. The latter enabled us to estimate cell-mediated cytotoxicity in a single cycle, which confirms the protective effect of interferon (IFN) after target cell treatment. This anti-cytolytic resistance is detected best by microassociation when effector and target cells are separately treated with IFN and associated thereafter. We suggest that, in inflammatory areas, enhanced cytotoxic activity of the IFN-treated effector cells is only operative before the establishment of protection in the targets, which is somewhat slower and appears in about 18 hr. Resistance of the targets could be at least partly attributed to the incapacity of effector cells to bind to them.

INTRODUCTION

The role of different interferons (IFN) in the regulation of immunological functions is complex and difficult to analyse. IFN can induce both enhancement (Svet-Moldavsky & Chernyakovskaya, 1967) and inhibition (Moore, White & Potter, 1980; Trinchieri & Santoli, 1978) of cell-mediated cytotoxicity (CMC). In general, during IFN treatment, immunological processes involving cell proliferation are inhibited, probably because of the known anti-cell growth effect of this substance (Paucker, Cantell & Henle, 1962). Established effector cell activity is more difficult to explore. Numerous authors have reported an enhancement of T-cell-mediated cytotoxicity by IFN (Heron, Berg & Cantell, 1976; Lindahl, Leary & Gresser, 1972). On the contrary, IFN-treated target cells have been found to be resistant to T-cell-induced cytotoxicity (Bergeret, Grégoire & Chany, 1980; Moore *et al.*, 1980; Trinchieri & Santoli, 1978). When the chromium release test is employed, this resistance becomes only apparent when every target cell is simultaneously hit by at least one truly sensitive T lymphocyte. The killer cell enumeration assay, where single effectors are directly associated to targets, enabled us to study the cytolytic changes in a single cycle. The results of these combined methods fully support the protective role of IFN against cellular cytotoxicity, as previously described (Bergeret *et al.*, 1980).

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MATERIALS AND METHODS

Cells and media

Mouse L1210 cells, maintained routinely in culture in RPMI 1640 medium containing 10% foetal calf serum (FCS), were used as targets.

Immunization procedure

Two-month-old C57 Black 6 mice were immunized by a single injection of 10^7 L1210 cells i.p. About 1 month later, spleens were removed and splenic lymphocytes were incubated for 5 days in RPMI 1640 medium in plates containing twenty-four flat-bottomed wells. Cells (5×10^6) were added to each well and mixed with an equivalent number of *in vitro* X-ray irradiated (2000 rad) BALB/c mouse splenic lymphocytes.

Interferon

Mouse IFN was prepared on a confluent layer of EAT cells (Van Venrooij, Henshaw & Hirsch, 1970) infected with Sendai virus (E72 strain) diluted in minimal essential medium (MEM), without serum, supplemented with theophylline 1 mM, according to a method described by Slattery, Taira, Broeze & Lengyel (1980).

Mouse IFN (sp. act. 3×10^{-6} u./mg of protein, titrating 6×10^6 international reference units—i.u.) was assayed using a monolayer culture of mouse L929 cells.

Statistical calculation

For all statistical calculations, we employed the method of paired comparisons by calculating whether the mean of the difference ($\bar{x} - y = m$) was significantly different from zero or not. The t value was calculated for small samples, using the formula $t = m/(s/\sqrt{n})$ where s = standard deviation and n = number of samples.

Cell-mediated cytotoxicity

Cell-mediated cytotoxicity (CMC) was assayed by the standard ^{51}Cr release test (Brunner, Mauel, Cerottini & Chapuis, 1968) to measure cytolytic activity of effector cell suspensions, and by the killer cell enumeration test (Zagury, Fouchard, Morgan & Cerottini, 1980).

In the ^{51}Cr release test, effector cell:target cell (E:T) ratios of 100:1 to 0·1:1, and incubation periods of either 4 or 18 hr were used. Each reaction was performed in five flat-bottomed microplate wells. Because of the importance of the spontaneous lysis in 18 hr, the results were expressed according to the formula: [(^{51}Cr release in presence of immune lymphocytes) − (^{51}Cr released in presence of control lymphocytes)/(total incorporation)] × 100.

Killer cell enumeration

The killer cell enumeration was performed by direct microassociation of a single effector cell to a single target cell and followed up during a 5–6 hr incubation period, as described previously by Zagury *et al.*, 1980 (Fig. 1). Killer cells were identified by lysis of their associated targets, while control effector cells did not induce cytotocity under the same experimental conditions. Lysis was appraised by the loss of cell refrin-

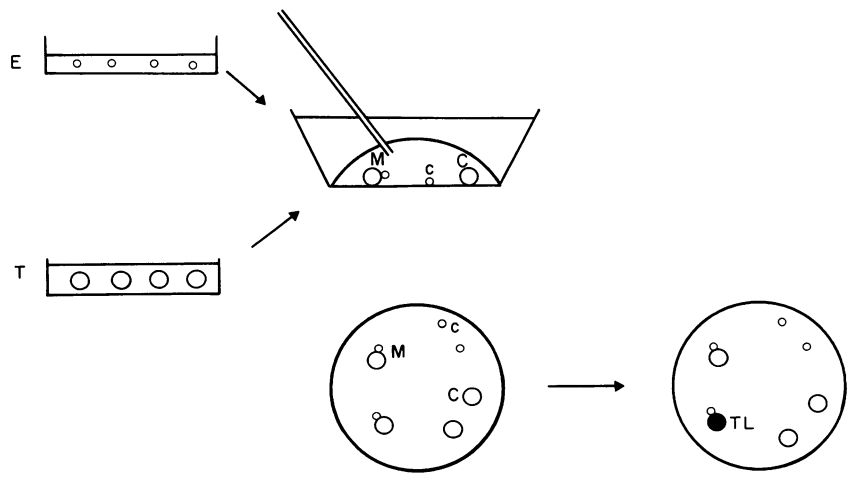

Figure 1. Direct enumeration of killer cells by single cell assay. *In vitro* resensitized effector cells are directly associated with the target in a microdrop using a Terasaki microplate. The killer cells are identified by the associated target lysis, detected by the loss of cell refringency. E, effector cell; T, target cell; M, microassociation of a T cell and an E cell; TL, lysed target showing loss of cell refringency; C, controls

gency under phase microscopy. The results were expressed, for each hour, as the percentage of cytotoxic cells/total cell population. In addition to lytic activity, a possible dissociation of effector cells from their targets was followed up during incubation.

RESULTS

In all experiments, cytotoxic assay was performed by microassociation, in parallel with the ^{51}Cr release test. Thus, in each case, the number of truly cytotoxic effector cells could be directly estimated.

Cytolytic T lymphocyte activity

Influence of the treatment of the target cells by mouse IFN. Target cells were treated by murine IFN (6×10^4 i.u.) for 18 hr at 37°. Cells were then centrifuged, suspended in MEM + 10% calf serum, labelled by ^{51}Cr (200 μCi), and cytotoxicity assayed as described above. Pretreatment of the target cells with mouse IFN significantly inhibited cytotoxicity, for E:T ratios varying from 100 to 0·1 (Table 1).

Influence of simultaneous treatment of effector cells and target cells by murine IFN. Effector cells were treated for 3 hr before the experiment with 6×10^3 i.u. of murine IFN at 37°. Then they were washed twice and incubated with IFN-treated target cells. As shown in Table 2, the protection against cytotoxicity induced by the sensitized and IFN-treated effector cells is clearly observed and comparable with the previous experiments where the effector cells were untreated.

Killer cell enumeration

Influence of the treatment of target cells by mouse IFN. As shown in Fig. 2, a significant delay in the appearance of cytotoxicity was observed after IFN treatment of the target cells. During a further incubation period, the recruitment of cytotoxic cells increased significantly slower than in the controls. The ^{51}Cr release test performed in parallel showed enhanced protection.

Influence of simultaneous and separate treatment of effector cells and target cells by mouse IFN. The simultaneous but separate treatment of effector and target cells by mouse IFN associated after careful washing of these cells constantly showed a highly significant decrease of the number of cytotoxic cells (Table 3). Thus, the final outcome of the cytotoxic effect is target-cell-dependent and clearly observed in spite of the somewhat increased cytolytic activity of the effectors.

Separation of previously associated IFN-treated effector target cells. Dissociations of doublets occurred in several assays (Table 3). This phenomenon was particularly clear in the course of the simultaneous treatment of effector and target cells performed in experiment 3, where all the cell doublets were dissociated after 3 hr of microscopic observation.

Table 1. Variation of the percentage of cytotoxicity in ^{51}Cr release test at different effector:target cell ratios after IFN treatment of the target cells

Exp. no.	Time of incubation (hr)	Effector:target cell ratios				
		100:1	40:1	10:1	1:1	0·1:1
1	4	−5·49	−2·31	−10·91	−1·72	−2·63
	18	−10·1	+0·18	−8·13	−6·71	+1·52
2	18	−6·39	−6·77	−4·48	−1·09	+3·93
3	18	−18·19	−15·89	−22·38	−20·59	−2·39
4	18	+2·54	+2	−16·36	−2·51	−3·95
5	18	ND	−0·77	−10·84	−21·23	−4·98
6	18	−14·05	−21·99	−8·65	−15·98	−1·31
7	18	−17·21	−17·31	−11·41	−20·96	−20·81

Significant decrease of cytotoxicity of sensitized lymphocytes after IFN treatment of the target cells ($P < 0·01$).
ND, not done.

Table 2. Variation of the percentage of cytotoxicity in ^{51}Cr release test at different effector:target cell ratios after parallel IFN treatment of effector and target cells.

Exp. no.	Time of incubation (hr)	Effector:target cell ratios				
		100:1	40:1	10:1	1:1	0·1:1
1	18	+0·19	+1·56	−2·2	−7·88	−11·22
2	18	ND	+0·46	−17·06	−22·42	−5·67
3	18	−11·83	−28·62	−14·17	−13·51	−7·27
4	18	−20·02	−21·25	−4·85	−10·34	−20·81

Significant decrease of cytotoxicity of sensitized lymphocytes after IFN treatment ($P < 0·01$).
ND, not done.

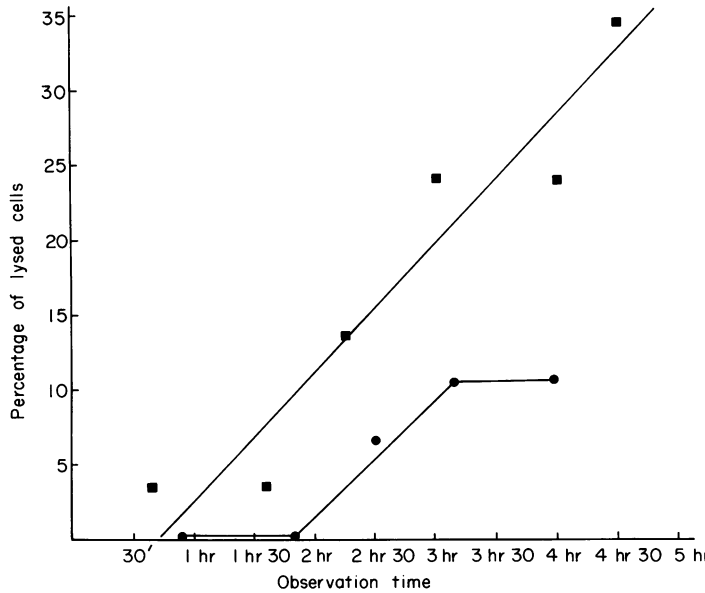

Figure 2. Percentage of lysed cells after interferon (IFN) treatment, or not, of the target cells (microassociation assay). (■) L1210 control cells; (●) L1210 cells + IFN.

DISCUSSION

Using the ^{51}Cr release assay, we have previously reported that IFN treatment of target cells reduces specific CTL activity against allogeneic sensitized cells (Bergeret *et al.*, 1980). In the present work, we confirm and extend further these results by CMC study at the single cell level. Direct association of effector and target cells enables us to estimate the number of cytolytic T lymphocytes (CTL) killers. This assay provides a single cycle analysis by excluding any possible transfer of the cytotoxic effector cell to a new target. With the ^{51}Cr assay, comparable conditions are obtained only with high E:T ratios (100:1 to 30:1), when target cells are simultaneously hit by at least one truly cytotoxic effector cell.

The inhibitory effect of T-cell-mediated cytotoxicity is detected both in the microassociation and in the parallel ^{51}Cr release tests. Even when the target and effector cells are separately incubated with IFN and

Table 3. Percentage of lysed cells at indicated time intervals after parallel IFN treatment of effector and target cells, or not (test performed by microassociation assay)

Exp. no.	Treatment of cells	Observation time (hr)					
		1	2	3	4	5	6
1	A	ND	25	25	31·25	ND	37·5
	B	0	0	0	14·28	ND	ND
2	A	8·82	11·76	ND	20·58	26·47	26·47
	B	4·58	12·5	ND	22·5	20·41	ND
3	A	ND	7·69	15·38	20·51	20·51	20·51
	B	ND	5·55	11·11	0*	0*	0*
4	A	4·16	20·83	29·16	33·33	33·33	33·33
	B	ND	13·33†	25	25	ND	ND

A, control lymphocytes/L1210 cells.
B, lymphocytes + IFN/L1210 cells + IFN.
ND, not done.
* Separation of all the cells.
† Three separations.

reassociated thereafter, the inhibition of target cell cytotoxicity is significantly and repeatedly found. In this reaction, the outcome of E–T interaction seems thus to depend more on the target than on the effector cell.

Since this experimental model is the closest to the events which might take place either during the IFN treatment of animals or during natural infections, it can be suggested that under *in vivo* conditions IFN inhibits T-cell-mediated cytotoxicity. This is in agreement with independent observations on NK cells by Trinchieri & Santoli (1978) and Moore *et al.* (1980). The increased cytotoxicity of separate and short IFN treatment of the effector T cells generally reported in the literature (Lindahl *et al.*, 1972) might play a role during early phases of natural infections, since anti-cytotoxic protection of the target cells appears only after at least 18 hr of incubation (Bergeret *et al.*, 1980). It could perhaps be operational in the case of IFN resistant targets.

A possible mechanism of IFN induced protection against CTL could be attributed to the observation that in some instances initially bound effector–target doublets separate before the administration of the lethal hit. It is well known that IFN induces a number of modifications in the constitution of the cell membrane, such as the synthesis and/or distribution of different transplantation antigens (Lindahl *et al.*, 1974). Since T-cell cytotoxicity is H_2-restricted, modifications of the synthesis of transplantation antigens can be taken into account. Moreover, the results here obtained by *in vitro* experiments are in line with experiments performed *in vivo*. Isoprinosine, a T-cell modulator, enhances indeed both the antiviral and anti-tumour effect of IFN only when injected before the substance. When injected simultaneously or after IFN, the enhancing effect decreases or disappears completely (Cerutti, Chany & Schlumberger, 1979).

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