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Interferon as a macrophage activating factor. I. Enhancement of cytotoxicity by fresh and matured human monocytes in the absence of other soluble signals

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SUMMARY

The cytolytic activity of human peripheral blood monocytes *in vitro* against K-562 human leukaemic target cells was stimulated by human fibroblast (β-) and leucocyte (α-) interferon (IFN). Stimulation was by up to several times the corresponding control activity, and was observed with freshly isolated monocytes, and with monocytes cultured for various periods up to 10 days. The cytolytic activity of untreated monocytes was detectable at very low effector:target ratios ($<5:1$), and fell between days 1 and 4 in culture, normally rising again towards the initial activity at day 8; this pattern was also observed when IFN was present continuously, although the activities were then always higher than in the corresponding control cells. Cytolysis showed a lag of about 6 hr, in contrast to that by natural killer (NK) cells, and was routinely measured over 24 hr. The course of stimulation by IFN and its dose–response were studied. Stimulation required the presence of IFN for at least 24 hr, and was maximal with between 1,000 and 10,000 units of IFN/ml. When IFN containing media were removed and replaced with control media, the monocyte activity remained stimulated for at least 4 days. Stimulation by β-IFN was blocked by a specific antibody to β-IFN, under conditions in which assayable IFN activity was also neutralized. Several control experiments indicated that the action of IFN was on the monocytes and not on the target cells. The morphological maturation of monocytes was retarded by IFN, even in cultures containing up to 50% serum. The effectiveness of fibroblast IFN indicated that stimulation could not be attributed to the lymphokines which might contaminate α-IFN. The action of IFN did not require simultaneous or antecedent *in vitro* stimulation by endotoxin. This was indicated both by serum free experiments, and also by others in which polymixin B was used to complex with and render unavailable any endotoxin present. Endotoxin showed an independent stimulatory effect, which could be prevented by polymixin.

INTRODUCTION

Interferon (IFN) is well known to activate mononuclear phagocytes in several respects (Nelson, 1976; Forster & Landy, 1981). Perhaps the most notable amongst these are spreading, Fc receptor expression, phagocytosis and tumoricidal activity (Schultz, Chirigos & Heine, 1978; Boraschi &

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Tagliabue, 1981). IFN can also restrict virus growth in macrophages (Virelizier, Allison & DeMaeyer, 1977).

More recently IFN has been frequently reported to positively regulate cytolytic activity of natural killer (NK) cells *in vivo* and *in vitro* (Bloom, 1980; Vilcek, Gresser & Merigan, 1980). In addition there is evidence that freshly isolated human peripheral blood monocytes possess cytolytic activity which is somewhat selective for transformed cells (Hammerstrom, 1981) and particularly effective against adherent cells (Gerrard, Terz & Kaplan, 1980). This activity of fresh monocytes can be stimulated by IFN (Jett, Mantovani & Herberman, 1980). The cytolytic activity of monocytes declines in culture, at least up to 2 days (Hammerstrom, 1979a). This decline parallels the morphological and functional 'maturation' (including cell expansion, and increased vacuolation and lysosomal enzyme activities: Lee & Epstein, 1980) which the monocytes undergo during this period. Since IFN has been found to retard both the morphological and biochemical *in vitro* maturation of monocytes (Lee & Epstein, 1980), it was the purpose of the present investigation to study cytolytic activity of monocytes during prolonged *in vitro* cultivation in its presence. It was anticipated that IFN might stimulate cytolysis mediated by both fresh and matured monocytes, and this proved to be the case.

Two other questions were immediately relevant. Firstly, it was necessary to consider the role of possible contaminants in the IFN preparations. Since lymphokines are also known to stimulate monocyte cytolysis (Hammerstrom, 1979a), we used both leucocyte IFN (which may be contaminated with lymphokines) and also fibroblast IFN (which should not be). In addition we provide evidence that IFN was the active factor in the fibroblast IFN preparations by experiments with an inhibitory antibody to IFN.

Secondly, since bacterial endotoxin (lipopolysaccharide, LPS) is also a potent acute stimulator of monocyte cytolytic activity (Hammerstrom, 1979b) we examined its role as a simultaneous or antecedent co-stimulator with IFN.

MATERIALS AND METHODS

Materials. Human 'buffy coat' residues from single donors were obtained from the Blood Transfusion units at Hopital Necker, Hopital St Antoine & Centre National de Transfusion Sanguin.

Human α-IFN was Sendai-induced human leucocyte IFN (Institut Pasteur, Paris) in ampoules containing 10^6 international units (iu) of IFN in 1 ml; it was stored at 4°C.

Human β-IFN was poly-IC-induced human fibroblast IFN, from the Rega Institute, Louvain, Belgium, in ampoules containing 5×10^6iu of IFN in 1 ml.; storage was at -20°C.

Rabbit antiserum to human β-IFN, a kind gift from Dr Billiau, Rega Institute, Louvain, was found to have a titre of 75,000 against 24 iu of human β-IFN, but had no neutralizing activity against human α- or γ-IFN.

Culture media and sera were from Eurobio, Paris & GIBCO (fetal calf serum, FCS, batch L2911020D) respectively. Ficoll was from Pharmacia. Endotoxin (LPS) was LPS W (*E. Coli* 0111:B4) from DIFCO (batch 652888). Polymyxin B sulphate was from Pfizer, Paris. Bovine serum albumin (BSA) was from Sigma, and the antibody to it was a kind gift of T. Pham Hu, Unite d'Immunohematologie, Hopital des Enfants Malades. ^{51}Cr was obtained weekly. Other reagents were of the highest quality commercially available.

Isolation of monocytes from buffy coats from single donors. This was performed as described previously (Taichman, Dean & Sanderson, 1980), using dextran sedimentation to remove erythrocytes, Ficoll gradients to obtain a mononuclear cell band, and selective adherence to obtain a monocyte rich population of cells. Adherence was normally in RPMI 1640 medium without serum, and with antibiotics (penicillin 100 iu/ml & streptomycin 100 μg/ml), for 1 hr, and was followed by four washes with Hank's balanced salt solution (HBSS) and overnight culture with RPMI without serum. Purity was then 85–95%. The medium was then changed to RPMI/10% heat-inactivated fetal calf serum (HIFCS) and cells matured after 4 days were >95% monocytes. For higher purity of fresh cells, adherence was in the additional presence of 10% HIFCS (Johnson,

Mei & Cohn, 1977). Purity was judged by morphology, latex phagocytosis in 4 hr, Giemsa staining, and in some experiments by non-specific esterase staining. Viability was always >90% as judged by trypan blue exclusion.

Cultivation of monocytes. Two different systems were used. In the first ('system 1'), 0·2 ml mononuclear cells ($2{\cdot}5 \times 10^6$/ml) was placed in each well of a flat bottomed Falcon 96 Multiwell plate (Microtest 2, 3042, Falcon, California, USA). After adherence (day '0') cells were gently washed with HBSS. Each wash comprised removing the medium, replacing it once and removing it again. Cells were kept overnight in fresh medium of the same constitution as that in which adherence had proceeded, and then the medium was changed the next morning (day '0'). Subsequent medium changes were at day 5, then at 2 day intervals, except were experiments required earlier medium changes. Unless otherwise stated, the media subsequently comprised RPMI/10% HIFCS with antibiotics.

In system 1 there was a progressive and variable loss of cells (up to 30% loss by day 5 as also reported by Johnson *et al.*, 1977), so that when target cells were added at various subsequent times the effector cell: target cell ratio varied from culture to culture. The numbers of cells remaining in certain experiments were determined by counting defined areas. The loss of cells was not affected by the presence of up to 2,500 iu/ml IFN (leucocyte or fibroblast). In general, system 1 gave $<7 \times 10^4$ monocytes/culture at day 0 (after washing), and $<5 \times 10^4$ at day 5 (after the day 5 medium change).

To avoid the problem of variability of monocyte number entailed by system 1, a larger scale cultivation system ('system 2') was also used. Whereas in system 1 cells remained *in situ* for the cytolysis assay, in system 2 they were harvested and then aliquoted at standard concentrations into the cytolysis plates. Thus in system 2, adherence was in 6 cm diameter culture dishes (8 ml of mononuclear cells at $2{\cdot}5 \times 10^6$/ml), followed by four washes with HBSS. Subsequent cultivation was as described for system 1. In both systems, cultures were maintained at 37°C in a humidified incubator gassed with 5% CO_2 in air.

Maintenance and labelling of target cells. K-562 cells, which are malignant erythro- and myelopoietic human stem cells, were grown in RPMI/10% HIFCS with antibiotics, in 75 cm^2 Falcon flasks. They were subcultivated every 2 days. Cells for use in a cytolysis assay were centrifuged and incubated at $3{\cdot}5 \times 10^6$ cells/ml with 100 μCi/ml ^{51}Cr, for 1 hr at 37°C. After this they were washed three times, incubated for 15 min in medium and washed once more. They were then counted and added to cytolysis assays.

Assay of cytolysis. With system 1 monocytes, target cells were added *in situ* into a final volume of medium of 200 or 250 μl, containing 10% HIFCS. System 2 monocytes were resuspended by scraping with a rubber policeman, counted and adjusted to 10^6 trypan blue excluding cells per ml. One hundred microlitres of the suspension was dispensed into each well of a microtitre plate before addition of targets (in a further 100 μl). The vast majority of monocytes were adherent within 1 hr of dispensing.

After incubation for 24 hr (except where otherwise stated), all the cells were in proximity to the plastic base, and the cell free supernatant was sampled directly for released ^{51}Cr. Experiments routinely included targets alone ('spontaneous release') at 5×10^3, 10^4 and 5×10^4 cells/ml in quadruplicate. Spontaneous release was measured for every duration of cytolysis used; after 24 hr it was never more than 20%. Monocyte mediated % specific release was computed as:

$$\frac{100 \times (\text{released chromium of monocytes} - \text{release by targets alone})}{(0{\cdot}85 \times \text{total radioactivity in system}) - (\text{release by targets alone})}.$$

Eighty-five percent of the total radioactivity in the system was taken as the maximum amount releasable, since this was the maximum released by Triton (0·1%), 0·5 N NaOH or 0·5 N HCl. Cell-mediated release never reached this level. As noted in many previous studies, the absolute number of cells killed was a complex function of the number of targets presented; the modifying effects of IFN were nevertheless qualitatively consistent.

Reproducibility of replicate assays in system 2 was good (s.d. <9% of the mean for $n=4$); in system 1, variation of effector cell numbers in the culture wells, especially after prolonged culture, contributed additional variability (s.d. <31% of the mean for $n=4$). Standard deviation bars are

shown on the figures, except where they are smaller than the symbol. There were pronounced quantitative differences in the behaviour of different preparations of monocytes from different individuals, as often described in the literature; and unlike the behaviour of mouse peritoneal macrophages pooled from many animals. The qualitative features described here were consistent however; and every experiment was conducted on cells from at least two different buffy coats, and at least twice with each culture system.

Assay of interferon activity. Anti-viral activity titration was performed as previously described (Virelizier, Lenoir & Griscelli, 1978) with slight modifications. The IFN titre was the reciprocal of the last dilution of the suspension tested that protected 50% of the cultures of MRC-5 human fibroblasts against the cytopathic effect of 100 infectious doses of a cloned variant of vesicular stomatitis virus giving large plaques (a kind gift from Dr J. C. Guillon, Institut Pasteur, Paris) in microplates (Falcon). One unit in our tests was equivalent to 1 international unit as judged by the standard IFN reference 67/87 (National Institute for Biological Standards and Control, London). The anti-viral activity of the samples was measured both before and at the conclusion of this work. Values given in this paper are based on the later assays, and thus may slightly (<25%) underestimate the activities present in the experiments.

RESULTS

Characterization of the assay and the target cells

The effect of the presence of endotoxin (1–10 ng/ml, 10–100 μg/ml) and IFN (both leucocyte and fibroblast: 100–12,500 iu/ml) on spontaneous release in 24 hr of ^{51}Cr from pre-labelled target K-562 cells (10^4/culture) in the absence of monocytes, was first studied. No significant effects were detected.

In other experiments, K-562 cells were pre-incubated in normal subculture conditions with 840 iu/ml β-IFN or without IFN, and then labelled and used in cytolysis assays with normal fresh

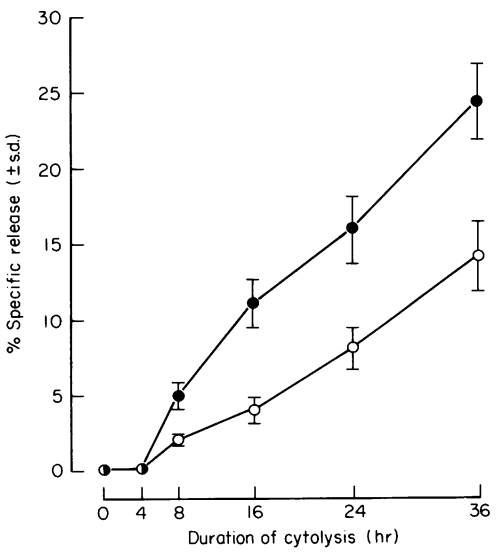

Fig. 1. The kinetics of cytolysis of K562 cells by human monocytes. Human monocytes on day 3 of cultivation by system 1 (described in Methods) received 10^4 K-562 targets in Falcon multiwell plates. Control cells were in the medium described in Methods, and IFN treated received 840 iu/ml of β-IFN. Chromium release at 0 time and after 4 hr of incubation was measured after centrifuging the plates (2,000 *g* for 10 min) to ensure that both effector and target cells were excluded from the sampled supernatant. This was unnecessary at later times; at which centrifugation was without effect on the measurements.

monocytes. Cytolysis was not significantly affected by this manoeuvre. Together, these preliminary experiments indicate that the effects of IFN reported subsequently are likely to operate on the monocytes rather than the target cells. In some experiments polymixin B increased 'spontaneous release' slightly, but this was not studied in detail.

Release from pre-labelled K-562 cells loaded onto the residue of layers of fresh monocytes killed by freezing and thawing four times was also compared with control spontaneous release. An inhibition of up to 20% by the monocyte material was observed with 10^3 targets/culture. This may have been due to physical entrapping of isotope, or to stabilization of the K-562 cells by the membraneous debris: similar stabilization has been reported previously. Because of these considerations target cell numbers of 5×10^3–5×10^4 were used even though with system 1 this did not permit a high effector:target ratio.

The kinetics of cytolysis by monocytes were also studied (Fig. 1). There was a lag of approximately 6 hr before significant specific release was detected; after this lag, cytolysis could be detected in 2 hr periods. This lag was not clearly altered by the presence of IFN during cytolysis, although the subsequent extent of cytolysis was increased. The kinetics of cytolysis by fresh and 4 day old monocytes prepared by method 2 were very similar. Neither the kinetics of spontaneous release, nor its extent at 24 hr were altered by IFN.

Cytolysis by Monocytes after various periods of cultivation in vitro

Fig. 2 indicates that the cytolytic activity (24 hr assay) during cultivation by method 2 of monocytes from a single donor. At all times the cytolytic activity was enhanced in the presence of IFN. The activity was substantial with fresh monocytes, dropped by day 3, and subsequently rose again. Fig. 2 also shows data for monocytes maintained continuously in the presence of IFN prior to assay in the absence of IFN. The drop and subsequent rise in cytolytic activity is qualitatively normal, although not universal. Monocytes cultivated with IFN are at all times more active than their control counterparts when assayed in the absence of IFN.

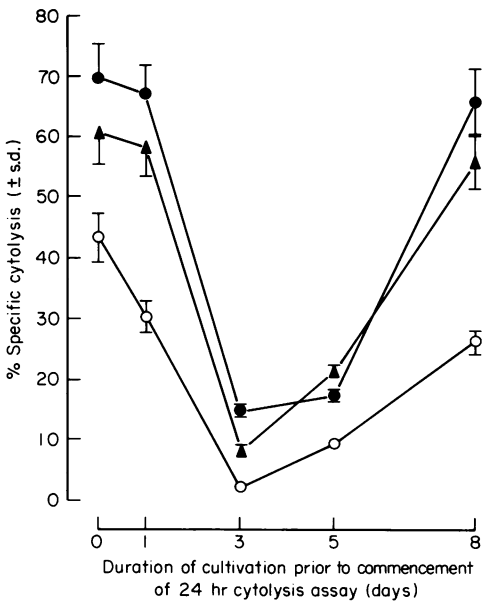

Fig. 2. Cytolytic Activity of human monocytes after cultivation for various periods (up to 8 days) with or without IFN. Cytolysis was assayed using monocytes prepared by system 2. The effector to target ratio was 10:1 (10^4 K-562 targets). Monocytes were pre-cultivated in either normal conditions or in the continuous presence of 840 iu/ml of β-IFN (which was added to the medium at each medium change). Cytolysis was then measured either in the absence of added IFN, or with 840 iu/ml β-IFN added. Control pre-cultivation = O (no addition at cytolysis); ● = + IFN at cytolysis; β-IFN pre-cultivation = ▲ (no addition at cytolysis).

Table 1. Dose–dependence of stimulation of monocyte cytolysis by IFN

Condition during cytolysis	% Specific release (±s.d.)
Control	33·7±2·9
+42 iu/ml β-IFN	35·0±3·1
+420 iu/ml β-IFN	51·0±4·1
+840 iu/ml β-IFN	55·0±4·2
+1,250 iu/ml α-IFN	53·2±4·1
+2,500 iu/ml α-IFN	57·9±4·6
+5,000 iu/ml α-IFN	69·1±4·8
+12,500 iu/ml α-IFN	64·3±5·1

Monocytes obtained by system 2 received 10^4 K-562 targets, on day 8. Effector:target ratio 10:1; cytolysis was conducted in the presence of various concentrations of IFN.

Dose–response and time course of stimulation of cytolysis by interferon

Dose–responses were studied with day 1, day 4 and day 8 cells (Table 1). Responses were quite similar, although the maximum response was obtained at different IFN concentrations (1,000–10,000 iu/ml) with cells from different buffy coats. Fibroblast and leucocyte IFN were comparably effective.

The time course of the cytolytic reaction itself has been described already. In order to further characterize the kinetics of the response of monocytes to IFN, batches of cells from a single donor were incubated with IFN for varying periods of time (1, 4, 12, 24 or 36 hr) prior to the cytolysis assay conducted without addition of further IFN. As shown in Fig. 3, cells pre-incubated for 24 hr or more showed enhanced cytolytic activity, and enhancement was maximal with approximately 24 hr pre-incubation.

Following this, the duration of enhanced cytolysis after a 24 hr pulse of β-IFN (840 iu/ml) was studied. After the pulse, the media were changed to normal without added IFN, and cytolysis assays (no further addition of IFN) were performed immediately, or after 1, 2, or 4 further days of

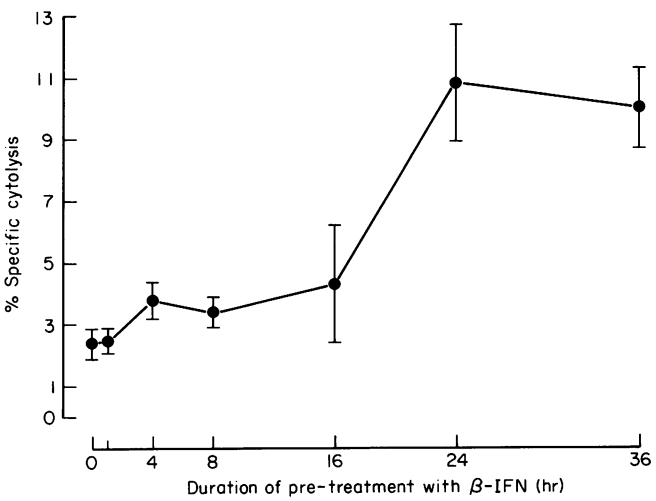

Fig. 3. Cytolytic activity of monocytes after various periods of exposure to β-IFN. Monocytes prepared by system 1, were pre-incubated with 840 iu/ml. of β-IFN for the specified period prior to a standard assay of cytolysis in the absence of added IFN, at day 2. Effector:target ratio <5:1, 10^4 targets (K-562).

Table 2. Inhibition by an antibody to β-IFN of stimulation of monocyte cytolysis by β-IFN

Normal condition during cytolysis	Measured IFN titre of medium at conclusion of cytolysis (iu/ml)	% Specific release (±s.d.)
Controls	<3	30·2±5·0
+840 iu/ml β-IFN	768	54·4±8·9
+anti-β-IFN	12	34·3±5·4
+840 iu/ml β-IFN+anti-β-IFN	36	35·3±5·6
+anti-bovine serum albumin (BSA)	<3	29·8±5·0
+bovine serum albumin	<3	29·4±4·8
+BSA+anti-BSA	<3	33·2±5·4
+840 iu/ml β-IFN+BSA+anti-BSA	576	52·5±5·4
+6250 iu/ml α-IFN	6,144	64·8±10·9
+6250 iu/ml α-IFN+anti-β-IFN	6,144	66·2±10·1

Monocytes ($\simeq 6 \times 10^4$) prepared by system 1 received 10^4 K-562 targets on dl. The media for use in the cytolysis were pre-incubated for 1 hr before commencement of cytolysis. The antibody to β-IFN was used in a nominal 10-fold excess over the β-IFN, and in the same quantity in the incubations with α-IFN. Where indicated, BSA was added at 100 μg/ml, and its antibody at previously determined equivalence.

cultivation (media were not changed again until the cytolysis assay commenced.). Cytolytic activity remained stimulated for at least 4 days (data not shown).

Confirmation that the stimulation of cytolysis is due to IFN: use of an inhibitory antibody

A specific antibody to human fibroblast IFN was used to neutralize the β-IFN preparation prior to presentation to monocytes. It largely suppressed the capacity of media containing β-IFN to stimulate cytolysis (Table 2). In parallel, we showed that the antibody abolished the IFN activity of the media assayable in a viral cytopathic assay (see Methods). This was shown both with media prior to presentation in monocytes, and with those recovered from the incubation with monocytes. Since antigen–antibody complexes (such as those resulting from the interaction of IFN with antibodies directed against it) themselves stimulate various actions of mononuclear phagocytes (see Nelson, 1976; Dean, Hylton & Allison, 1979; Dean 1980) control experiments with BSA, antibodies to BSA and with antigen–antibody complexes resulting from their combination in quantities somewhat greater than those of IFN–anti-IFN used, were performed. These control conditions did not affect monocyte cytolysis (Table 2). Neither antibody had significant effect on stimulation by α-IFN. Thus the blockade by specific antibodies of the stimulation by β-IFN preparations, provides strong evidence that the stimulation is due to IFN itself.

Is endotoxin required for the expression of stimulation by IFN?

Several functions of monocytes require dual stimulation, either simultaneously or successively: for instance, production and secretion of certain neutral proteinases (see Nelson, 1976). Commercial sera and proteins normally contain relevant amounts of bacterial endotoxins, which commonly function as co-stimulants in such systems: for instance endotoxin is required for lymphokines to stimulate cytolysis by mouse macrophages (Taramelli, Holden & Varesio, 1980). Therefore we investigated the possible involvement of endotoxin as a prerequisite for or synergist with stimulation by IFN. Endotoxin is known to stimulate monocyte cytolysis (Hammerstrom, 1979b) and this was confirmed (data not shown in detail: see Tables 3 & 4).

Firstly, monocytes were isolated and cultivated in the absence of serum and thus of exogenous endotoxin. As shown in Table 3 with day 3 monocytes, IFN could stimulate normally even cells cultivated without serum; this was shown also with day 1 and day 8 cells. However, the cytolysis itself was always conducted in the presence of serum (and thus of endotoxin), since spontaneous release was excessive in its absence. Thus secondly, polymixin B sulphate, which forms complexes with endotoxins, blocking their activity (e.g. Boraschi & Tagliabue, 1981) was used. Under

Table 3. Stimulation of cytolysis in monocytes pre-cultivated in serum-free conditions: lack of requirement for endotoxin for the effect of β-IFN

Condition	% Specific release (±s.d.)
Controls	4·3±0·7
+840 iu/ml β-IFN	10·2±1·8
+1,680 iu/ml β-IFN	17·8±2·8
+1 ng/ml LPS	4·1±0·7
+1 μg/ml LPS	11·3±1·7
+840 iu/ml β-IFN+1 ng/ml LPS	9·9±1·6

Monocytes prepared by system 1 were used for a standard cytolytic assay at day 2, in the presence of various stimuli. Ten thousand K-562 targets; effector:target ratio ≤5:1. LPS=endotoxin.

Table 4. Prevention of LPS stimulation of monocyte cytolysis by complex formation with polymixin B: further evidence that LPS is not required for stimulation by β-IFN

Cytolysis condition	% Specific release (±s.d.)
Control	15·0±1·6
+1 ng/ml LPS	13·0±1·4
+1 μg/ml LPS	38·1±2·9
+1 μg/ml polymixin B	12·8±1·4
+10 μg/ml polymixin B	16·5±1·9
+1 ng/ml LPS+1 μg/ml polymixin B	17·8±1·9
+1 μg/ml LPS+10 μg/ml polymixin B	20·4±1·9
+840 iu/ml β-IFN	39·9±4·2
+840 iu/ml β-IFN+10 μg/ml polymixin B	38·6±4·3

Monocytes (system 1, day 1) were used in cytolysis against 10^4 K-562, in the specified conditions. The effector:target ratio was ≏8:1.

conditions in which endotoxin activity on cytolysis was blocked by polymixin, added IFN was effective as normal (Table 4). There were indications that polymixin itself has a stimulatory effect on cytolysis but these were not studied closely. Thus both kinds of experiment indicated that endotoxin was not a necessary collaborator for the stimulation by interferon nor was it markedly synergistic with IFN.

The similarity of stimulation by α- and β-IFN largely excludes the possibility that lymphokines might be a necessary co-stimulant with IFN (since lymphokines do not contaminate β-IFN).

DISCUSSION

Monocytes can exert both cytostatic and cytolytic effects (Vilcek *et al.*, 1980). The latter were the subject of the present work. Previous studies (Jett *et al.*, 1980; Stanwick, Campbell & Nahima, 1980) have also indicated that monocyte cytolysis can be stimulated by IFN, but have almost solely concerned freshly isolated cells. The present data confirm such previous studies; reveal that cytolysis can be effected at low monocyte:target cell ratios (>5:1); and extend the observations to monocytes in various stages of *in vitro* maturation, which are shown to be stimulated similarly by

IFN. The stimulation correlates with the known retardation of morphological maturation in culture (Lee & Epstein, 1980). In our studies this retardation was particularly obvious in a few experiments in which monocytes were cultivated in the presence of high (50%) concentrations of serum, which accentuates the expansion of the control cells.

The purity of the adherent monocyte preparations makes it unlikely that the cytolysis reported was mediated by NK cells, a non-adherent cell type. This view is further supported by the distinctive kinetics of cytolysis, with a lag of approximately 6 hr before detectable chromium release occurs, followed by a rate of release which allows detection within subsequent 2 hr periods (as also noted by Horwitz *et al.*, 1979). Most workers have not studied the kinetics systematically, although several have noted negligible release in 4 hr assays (e.g. Jett *et al.*, 1980). Fischer, Hubbard & Koren (1981) reported an exception, with rapid cytolysis, using a system like system 2 herein. The reasons for this accelerated activity are unclear.

The mechanism by which IFN stimulates cytolysis remains unknown, as largely does the mechanism of killing by control monocytes. However, increasing evidence implies that killing involves free radical oxygen metabolites (Nathan *et al.*, 1979; Temple 1981), so that IFN may act on the pathways generating such metabolites. Since mononuclear phagocytes, tumour cells and lymphocytes can all produce IFNs, it seems likely that there are circumstances in which the stimulation of monocyte cytolytic activity against abnormal cells can constitute an important defence, supporting that of NK cells. However, recent evidence has also shown conversely that IFN may sometimes protect cells against cell-mediated lysis (Welsh *et al.*, 1980).

Our work shows that IFN enhances monocyte cytolytic activity without the cooperation of certain other soluble stimuli such as LPS and lymphokines. In this respect, IFN appears to have a direct 'macrophage activating factor' (MAF) activity. Thus IFNs, whatever their cellular source or molecular type, may be major MAF molecules, able to regulate, through their direct effects on mononuclear phagocytes, host responses to tumours and micro-organisms. This is in agreement with our previous finding that patients with selective defects of leucocyte IFN production suffer from immunoblastic lymphomas, and severe persistent viral or bacterial infections (Virelizier & Griscelli, 1980). The MAF activity of IFN should be considered in the pathogenesis and treatment of such human diseases.

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