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Effect of interferon on the induction of human monocyte secretion of interleukin-1 activity

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Summary. This study investigates the effect of interferons on the induction of human monocyte secretion of interleukin-1 (IL-1) activity by lipopolysaccharides (LPS). Monocytes do not spontaneously produce IL-1 and the addition of interferons to the culture does not lead to detectable secretion. Addition of LPS alone induces the release of measurable amounts of IL-1 activity. The addition of low doses (1–10 units/ml) of alpha, beta, or gamma interferon to the LPS-stimulated cultures further increases this secretion by 50%. The addition of 1000 units/ml of alpha or beta interferon leads to inhibition of IL-1 release. By contrast, gamma interferon is a dose dependent enhancer of IL-1 release. The effect of gamma interferon is on the production of IL-1 and is not an enhancement of IL-1 activity in the biological assay. Results demonstrate that addition of gamma interferon to monocytes increases the rate of secretion of IL-1 by these cells. Gamma interferon also appears to abrogate the loss in the ability of monocytes to produce IL-1 activity after overnight culture. This last result parallels the maintainence of the expression of the HLA-DR surface marker on monocytes by gamma interferon. These results may help define a mechanism involving IL-1 generation which could have bearing on the *in vivo* pyrogenic effects of purified gamma interferon.

Abbreviations: FBS: fetal bovine serum, IFN: interferon form gamma -γ, alpha -α or beta -β, IL-1: interleukin-1, LPS: lipopolysaccharide, PHA, phytohaemagglutinin.

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INTRODUCTION

Crude lymphokine supernates derived from stimulated T lymphocytes contain a number of biological activities which affect macrophage functions. Such materials have been demonstrated to enhance macrophage pinocytosis (Schubert, Wong & David, 1980) alter energy metabolism and superoxide formation (Higuchi *et al.*, 1983), increase oxidative metabolism and microbicidal properties (Nathan *et al.*, 1983), alter the expression of surface markers (Steeg, Moore & Oppenheim, 1980) and activate macrophage cytotoxicity against tumour target cells (Fischer, Golightly & Koren, 1983). Many of these activities have been attributed to a protein molecule which has been termed macrophage-activating factor. Recent studies have indicated that this activity is similar, if not identical, to gamma(IFN-γ) or immune interferon (Nathan *et al.*, 1983; Schreiber *et al.*, 1983).

While all of the activities listed above are not directly linked with IFN-γ effects, the evidence for the ability of IFN-γ to influence macrophage functions is substantial. Besides its role in the alteration of monocyte properties, IFN-γ appears to play a role in the

regulation of the immune system (Basham & Merigan, 1980). This role includes the regulation of monocyte Ia and DR surface antigen expression (Becker, 1984), regulation of T-cell differentiation (Farrar, Johnson & Farrar, 1981) and the regulation of natural killer cell activity (Ratliff, McCool & Catlona, 1981). Moreover, the regulation is not unidirectional and the production of IFN-γ itself is closely associated with the production of the T-cell lymphokine interleukin-2 (IL-2) (Pearlstein *et al.*, 1983). The production of IL-2 is in turn controlled by the monokine interleukin-1 (IL-1) (Gillis & Mizel, 1981). The association of the regulation of production of these three activities forms an interesting model for the study of cell interactions in immune responses.

The potential role of IFN-γ in the regulation of IL-1 production has not been investigated. A demonstration of the regulation of IL-1 synthesis by IFN-γ would either close a cycle of positive feedback regulation or provide the negative signal which feedback inhibits this sequence of reactions. Therefore, a study was undertaken to examine the influence of IFN-γ on IL-1 synthesis by human monocytes.

The results of this study demonstrate that IFN-γ is a dose dependent, positive regulator of monocyte IL-1 production. IFN-γ itself does not induce IL-1 secretion, but the addition of as little as 1 unit/ml of IFN-γ results in an enhancement of the IL-1 production induced by bacterial lipopolysaccharides. IFN-α and IFN-β also enhance secretion of IL-1 but only at the lower doses tested. A dose of 1000 units/ml inhibits IL-1 secretion with these two forms. The enhancement of LPS induced IL-1 production by IFN-γ appears to be due to an enhanced rate of release of IL-1. In addition, overnight incubation of monocytes with IFN-γ preserves the ability of monocytes to produce IL-1 in parallel with the preservation of the expression of the HLA-DR surface marker. Such results indicate that IFN-γ may be involved in the positive regulation of immune and non-immune responses in which IL-1 has been shown to play a role.

MATERIALS AND METHODS

Materials

RPMI 1640 medium was obtained from M. A. Bioproducts, Walkersville, MD. Pyrogen-free 0·15 M sodium chloride solution was purchased from Abbott Laboratories, Chicago, IL. Lipopolysaccharide extracted from *Salmonella minnesota* Re595 was obtained from Calbiochem Behring, San Diego, CA. Low endotoxin fetal bovine serum (FBS) was obtained from Sterile Systems, Logan, UT. Low endotoxin human AB serum, prepared from plasma, was obtained from Irvine Scientific, Santa Ana, CA. Affinity purified human IFN-γ was purchased from Interferon Sciences, Inc., New Brunswick, NJ. The specific activity of this material was $5{\cdot}2 \times 10^6$ units of activity per mg of protein. Pure human IFN-β and partially purified IFN-α were a generous gift from Dr E. Knight of Du Pont Central Research and Development Department. The IFN-α had a specific activity of 1×10^7 units per mg protein and the IFN-β 2×10^8 units per mg protein. Monoclonal antibodies were obtained from BRL, Gaithersburg, MD (61D3), and Becton Dickinson, Mountain View, CA (Leu-M3, HLA-DR).

Monocyte preparation

Monocytes were prepared from units of heparinized whole blood (Interstate Blood Bank, Philadelphia, PA) by centrifugal elutriation. Blood was diluted with 0·15 M saline containing 0·05% EDTA and centrifuged over Ficoll-Paque (Pharmacia, Piscataway, NJ). The interface cells were washed once with saline/EDTA and then passed over a gradient of Ficoll-Paque diluted with saline to a density of 1·058. The pellet was resuspended in RPMI 1640 containing 2·5% FBS and 0·05% EDTA and cells separated by centrifugal elutriation using a Beckman J2-21 centrifuge, a Beckman elutriation rotor, and a standard elutriation chamber. The separation was made using constant flow rate with varied centrifuge rotor speed. The populations isolated for use as monocytes consisted of 90% monocytes, 3% lymphocytes, 5% neutrophils, and 2% basophils and eosinophils as determined by Wright-Giemsa staining morphology.

Cell culture

Cells were plated at 1×10^6 cells per ml in RPMI-1640 medium containing 5% human serum. Cells were plated in Costar (Costar, Cambridge, MA) 12 well culture dishes with 1·5 ml cell suspension per well. For overnight cultures where cells were to be collected for surface marker analysis, the cells were plated in 60 mm Teflon resin dishes. All conditions were tested in duplicate. Unless noted otherwise, IL-1 was induced by the addition of 1 μg/ml LPS. After 16–20 hr of culture at 37°, the supernates were collected and assayed for IL-1 activity.

Endotoxin assay

All reagents were tested for endotoxin content using a colorimetric assay obtained from M. A. Bioproducts. The assay was performed in Costar 96 well flat-bottomed microtitre plates. Samples were assayed in duplicate and compared against a standard curve obtained using an endotoxin sample of *E. coli* 0111 : B4 LPS containing 11 endotoxin units/ng of LPS. Each well received 100 μl of chromogenic substrate, 50 μl of test sample and 50 μl of Limulus lysate. The plate was mixed and incubated at 37° for 10–15 min. The reaction was stopped by the addition of 100 μl of 50% acetic acid to each well and the absorbance of the wells measured spectrophotometrically at 405 nm.

IL-1 assay

The thymocyte proliferation assay was performed using thymocytes obtained from 5–7-week-old C3H/HeJ mice (Jackson Laboratory, Bar Harbor, ME) which were stimulated with 1 μg/ml of purified PHA (Burroughs Wellcome Co., Greenville, NC). Samples were serially diluted in duplicate in 100 μl amounts with RPMI 1640 containing 20% FBS. Thymocytes in 100 μl of medium containing 1×10^{-4} M 2-mercaptoethanol were added to each well. After 48 hr of culture the cells were labeled by the addition of 1 μCi/well [^{3}H]thymidine (6·7 Ci/mmol, New England Nuclear, Boston, MA) for 16 hr. The dilution giving 50% maximum label incorporation was calculated for each sample and compared to a standard containing 8 units/ml IL-1 activity (i.e., this sample gave 50% maximal incorporation at a 1/8 sample dilution). All data for IL-1 activity are presented in units/ml. Statistical evaluation of the results was carried out using the Student's *t*-test.

Surface marker analysis

Cells were removed from culture on Teflon resin dishes after 18 hr and washed three times with Dulbecco's phosphate buffered saline containing 0·02% sodium azide. Cells were counted and the number adjusted to 1×10^6 cells/ml. Cells were stained for surface 61D3, Leu-M3, and HLA-DR antigen expression using monoclonal antibodies in an indirect immunofluorescence assay with fluorescein labeled goat anti-mouse gamma globulin (Tago, Burlingame, CA) as the

Table 1. Effect of various levels and types of interferon on the induction of IL-1 secretion by human peripheral blood monocytes over the first 20 hr of culture

Interferon	Concentration (U/ml)	IL-1 (U/ml)*	SEM†	Percentage change‡	Significance§
None					
No LPS	—	0·0	0·0	—	**
LPS	—	26·4	1·8	—	—
IFN-α	1	40·5	2·5	+53	**
	10	44·0	3·0	+67	**
	100	26·7	2·3	+1	NS
	1000	18·8	3·4	−29	*
IFN-β	1	27·0	0·0	+2	NS
	10	38·0	3·0	+44	**
	100	29·2	0·8	+11	NS
	1000	5·6	2·8	−79	**
IFN-γ	1	31·9	1·6	+21	*
	10	45·7	2·2	+73	**
	100	60·3	1·9	+128	**
	1000	68·3	3·0	+159	**

Data are a summary of the results of six separate experiments.

* IL-1 activity expressed in units/ml induced by the addition of 1 μg/ml LPS except where noted.

† SEM = standard error of the mean.

‡ Calculated by $\frac{(\text{U/ml IF/LPS} - \text{U/ml LPS})}{\text{U/ml LPS}} \times 100$.

§ Level of significance as compared with no interferon, LPS added, sample: NS = not significant ($P > 0{\cdot}05$), * = $P < 0{\cdot}05$, ** = $P < 0{\cdot}01$.

second antibody. Cells were analysed using an Ortho Cytofluorograph System 50-HH (Ortho Diagnostics, Raritan, NJ)

RESULTS

Interferon effect on IL-1 induction by LPS

The normal production by human monocytes stimulated with 1 μg/ml LPS is shown in Table 1. Unstimulated cells do not secrete detectable IL-1 activity into the surrounding fluid. In the experiments conducted in this study, the addition of LPS induces the secretion of an average of 26 units/ml IL-1 activity by 1×10^6 monocytes in the first 20 hr of culture. The addition of 1–10 units/ml of either IFN-α or IFN-β yields about a 50% increase in the level of IL-1 activity secreted in the first 24 hr of culture (Table 1). However, at a level of 1000 units/ml IFN-α or IFN-β decreases the level of IL-1 activity released. IFN-α reduces the production of IL-1 activity by 29% while IFN-β decreases production by 79%.

In contrast, addition of as little as 1 unit/ml of IFN-γ results in an enhancement of IL-1 secretion by LPS stimulated cells. The enhancement increases with dose of interferon up to the highest dose tested (1000 units/ml). The increases in IL-1 secretion are highly significant and reproducible. All of the interferons in the range of the doses tested do not induce any detectable IL-1 secretion by monocytes in the absence of LPS addition. All the forms tested contained undetectable amounts of endotoxin as assessed by the assay employed which is capable of readily detecting in the range of 1–10 pg/ml of LPS. Finally, none of the interferons exhibits any appreciable effect on the thymocyte assay for IL-1 activity; i.e., the interferons exhibited no activity by themselves nor did they modify the activity of a standard IL-1 preparation in the assay.

Effect of interferon pulse on IL-1 induction

The effects of most interferons can be demonstrated with a short pulse of the target cells with interferon. The interferon binds to the cell through highly specific surface receptors (Joshi, Sarker & Gupta, 1982). To demonstrate that cells could be pulsed with interferon and demonstrate the same enhancing effect on IL-1 production, cells were pulsed by the addition of 100 units/ml of the three forms of interferon for one hour. The cells were then washed three times with medium and induced to secrete IL-1 by the addition of LPS. As seen in Table 2, there is again appreciable enhancement (68% increase) of IL-1 production with the addition of IFN-γ. A slight but significant increase (12%) is also seen with IFN-β but the amount of the increase is not as large or as significant as with IFN-γ.

Effect of interferon on LPS sensitivity of monocytes

A possible mechanism by which the IFN-γ could

Table 2. The effect of a 1 hr pulse of interferon on subsequent production of IL-1 by human monocytes stimulated for 20 hr by the addition of LPS

Addition*	IL-1 (U/ml)	SEM†	Percentage change‡	Significance§
None	0·0	0·0	—	**
LPS	40·3	2·0	—	—
LPS+IFN-α IF	38·0	4·2	−6	NS
LPS+IFN-β IF	45·0	1·6	+12	*
LPS+IFN-γ IF	67·6	4·3	+68	**

Data are a summary of the results of three separate experiments.

* Interferons were added at a level of 100 units/ml for 1 hr followed by three washes of the cells. LPS was then added at a concentration of 1 μg/ml.

† SEM = standard error of the mean.

‡ Calculated as in Table 1.

§ Level of significance as compared with no interferon, LPS added, sample: NS = not significant ($P > 0{\cdot}05$), * = $P < 0{\cdot}05$, ** = $P < 0{\cdot}01$.

Table 3. Effect of different doses of IFN-γ on the level of IL-1 activity produced by human monocytes in response to decreasing concentrations of LPS

LPS concentration	Interferon added							
	0 U/ml	SEM*	1 U/ml	SEM	10 U/ml	SEM	100 U/ml	SEM
None	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
10 ng/ml	17·5	0·5	25·5	0·5	35·0	7·0	51·5	3·5
1 ng/ml	13·5	1·5	16·0	0·2	29·0	1·0	40·5	5·5
100 pg/ml	13·0	0·2	16·0	1·0	25·0	3·0	23·0	2·0
10 pg/ml	<0·1†	0·0	0·2	0·2	2·8	0·3	2·7	0·2
1 pg/ml	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
100 fg/ml	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0

Data are the summary of the results of two separate experiments.
* SEM = standard error of the mean.
† Level of IL-1 activity unable to be calculated in units/ml. However, counts significantly above background measurable at 1/2 and 1/4 sample dilution.

enhance IL-1 secretion would be to enhance the monocyte ability to recognize and respond to the presence of LPS. To test whether the IFN-γ enhances the sensitivity of monocytes to the presence of LPS, monocytes were activated with decreasing amounts of LPS in the presence or absence of 1, 10, or 100 units/ml of IFN-γ. The results are presented in Table 3. Rather than seeing a significant shift in the threshold of LPS dose responded to, it appears that all levels of IL-1 induction by LPS are enhanced to a similar extent. The enhancing effect is seen at all doses of interferon tested across the range of LPS concentrations tested. These results indicate that IFN-γ does not enhance the monocyte sensitivity to the presence of LPS but amplifies the response to LPS. The effect of IFN-γ on the induction phase of IL-1 following LPS addition was next examined. Figure 1 demonstrates that IFN-γ dramatically enhances the rate of secretion of IL-1 in this phase of induction.

Effect on monocytes cultured overnight

Monocytes which have been cultured overnight decrease in their ability to support immune reactions (Mayernik, Ul-Haq & Rinehart, 1983). This culture period is marked by loss of the expression of surface HLA-DR antigens (Becker, 1984). As shown in Table 4, there is also a significant decrease in the ability to produce IL-1 in response to LPS addition. This

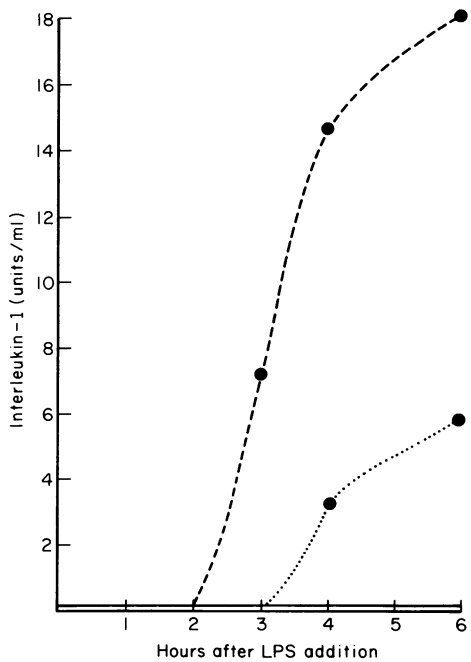

Figure 1. Time course of LPS-induced secretion of IL-1 by human monocytes in the presence or absence of IFN-γ. Cells were incubated for 6 hr with no additions (——), or in the presence of 1 μg/ml LPS with (– – –) or without (· · · · · ·) the addition of 100 units/ml γ interferon. Samples of supernate were collected and assayed for IL-1 activity at the appropriate time points.

Table 4. Effect of overnight culture after a 1 hr incubation in the presence or absence of interferon on the subsequent production of IL-1 activity induced by LPS

Additions*	IL-1 (U/ml)	SEM†
None, No LPS	0·0	0·0
None, LPS	<0·1‡	0·0
IFN-α, LPS	<0·1	0·0
IFN-β, LPS	<0·1	0·0
IFN-γ, LPS	22·0	2·6

Data are the summary of the results of two separate experiments.

* Monocytes were incubated for 1 hr in the presence or absence of 100 U/ml of different interferons. After a subsequent 18 hr incubation, cells were induced to secrete IL-1 by the addition of 1 μg/ml LPS for 24 hr. These cell populations released an average of 31 units/ml IL-1 in response to LPS over the first 20 hr of culture.

† SEM = standard error of the mean.

‡ Level of IL-1 activity unable to be calculated in U/ml. However, counts significantly above background measurable at 1/2 and 1/4 sample dilution.

Table 5. Expression of various surface markers after overnight culture of cells in the presence or absence of various interferons

Marker*	No addition	IFN-α†	IFN-β†	IFN-γ†
61D3	89·0%	90·4%	89·6%	87·8%
Leu-M3	87·8%	91·2%	91·1%	88·7%
HLA-DR	91·4%	90·4%	94·6%	94·0%

Data are the summary of three separate experiments and represent the total percent positive cells after 18 hr of culture.

* Cells were stained for surface markers as described in 'Materials and Methods'.

† 100 U/ml.

decrease cannot be overcome by the addition of higher levels of LPS (data not shown). Also shown in Table 4 is the fact that overnight incubation after a pulse with 100 units/ml IFN-γ results in a significant decrease in this loss of IL-1 inducibility. The addition of IFN-α or IFN-β does not show this effect. This result parallels the ability of the IFN-γ to maintain the expression of HLA-DR surface antigens (Fig. 2). Although there is a distinct influence on the level of expression, the total number of monocytes expressing HLA-DR as well as two other monocyte surface markers is not affected by the addition of IFN-γ (Table 5).

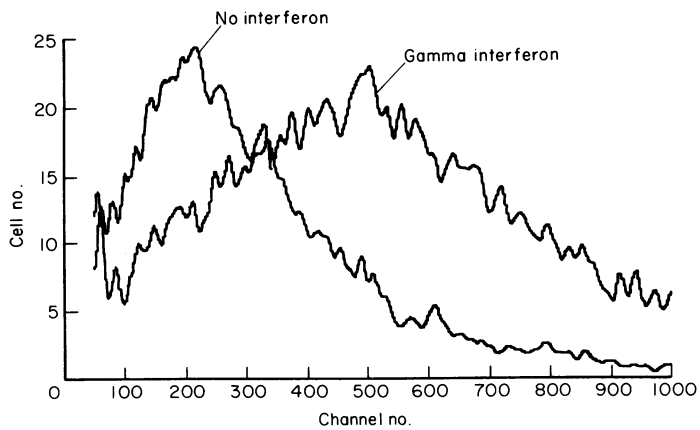

Figure 2. Fluorescent analysis of cells stained for HLA-Dr surface expression after overnight culture in the presence of interferon. Cells were incubated overnight in the presence or absence of 100 U/ml gamma interferon before staining for HLA-DR expression as described in 'Materials and Methods'. Overnight incubation with 100 U/ml α or β interferon gave results similar to the no interferon treatments.

DISCUSSION

Interferon was originally described by Isaacs & Lindemann (1957) as a molecule which conferred on cells the ability to resist viral infection. The gamma or Type II form of interferon was originally described by Wheelock (1965). This form of interferon is induced *in vitro* by the addition of antigens or mitogens to lymphocytes. A number of studies have shown a wide range of biological effects of IFN-γ on the immune response which appear to be unrelated to the induction of the antiviral state in cells. In fact, IFN-γ shows a number of differences from the other classes of interferon, IFN-α and IFN-β (Type I forms), in its effects on cells of the immune system. For example, IFN-γ alone has been shown to augment NK cell cytotoxicity (Ratliff *et al.*, 1981), increase Fc receptor expression on monocytes (Perussia *et al.*, 1983a), induce myeloid cell differentiation (Perussia *et al.*, 1983b) and induce increased histocompatability antigen expression (Basham & Merigan, 1982). Such evidence indicates that IFN-γ may play a more important role as a regulator of immune cell interactions then as an antiviral compound. The results presented here support this observation.

The studies presented in this paper detail the effect of IFN-γ on the induction of human monocyte secretion of IL-1 activity by bacterial LPS. Human monocytes when placed in culture do not spontaneously produce IL-1 activity, and the addition of 1–1000 units/ml of IFN-α, IFN-β or IFN-γ also does not induce detectable secretion (Table 1). This is in contrast to the results of other studies (Koretsky *et al.*, 1983, Trevese*t al.*, 1983) but is a highly reproducible finding in this laboratory. This difference is probably related to the strict control of LPS contamination of buffers and media. The addition of LPS induces these cells to secret easily measureable amounts of activity. The addition of interferons over this culture period gives varying results depending on the type and dose of interferon added (Table 1). In agreement with the results of other studies (Herman, Kew & Rabson, 1984) IFN-α and IFN-β appear to enhance the production of IL-1 at doses of 1 or 10 units/ml. The addition of higher levels of these interferons interferes with production with a dose of 1000 units/ml actually suppressing the production of IL-1. In contrast, IFN-γ demonstrates a dose dependent enhancement of the production of Il-1. The enhancement is not due to an effect on the IL-1 assay. This enhancement has been demonstratable in every experiment to date. Similar enhancement can be obtained with a one hour pulse of the cells with IFN-γ (Table 2).

The increased production of IL-1 activity induced by IFN-γ could be due to a heightened sensitivity of the cells to the presence of LPS. This influence would be exerted at the level of LPS triggering of IL-1 synthesis and secretion. Alternately, the presence of IFN-γ could influence the pathways involved in the synthesis of proteins in general or in the enhancement of cell secretion events. To test which of these mechanisms appeared to be the most likely, cells were stimulated with 1, 10 or 100 units/ml of IFN-γ in the presence of decreasing doses of LPS. As seen in Table 3, there appears to be an enhancement of IL-1 secretion at all levels where LPS alone induces detectable IL-1 secretion. The enhancement is similar at each dose of LPS and there does not appear to be an extension of the range of sensitivity to lower doses of LPS. The lowest level of LPS which shows any detectable induction of IL-1 secretion is 10 pg/ml. Lower levels of LPS, down to 100 fg/ml showed no induction of IL-1 with or without the addition of interferon. If IFN-γ were affecting the generation of cell signals that result from LPS binding which lead to activation of the IL-1 gene, a synergy of IFN-γ should occur with doses of LPS just below those which induce IL-1. This synergy is not seen at any dose of LPS below 10 pg/ml (the minimal triggering dose with LPS alone). These results indicate that the IFN-γ is probably acting through another mechanism which influences the production of IL-1 and is not related to the events which occur at the cell membrane upon LPS binding. Thus, IFN-γ does not enhance the monocyte sensitivity to the presence of LPS but amplifies the response to LPS. In support of this conclusion, IFN-γ enhances the rate of IL-1 secretion by monocytes (Fig. 1). While it may appear that secretion of IL-1 also occurs earlier than with LPS alone, the difference is quantitative. The results of three experiments have demonstrated that while the quantitative differences can be up to 15-fold after 3 hr with IFN-γ addition, there is no time point where secretion is seen by only one of the samples (data not shown). However, the discrimination of whether IFN-γ enhances expression of the IL-1 gene at the transcriptional or translational level is limited by the current methodology and awaits the development of sensitive assays in molecular biology. These data also do not rule out IFN-γ acting to increase the number of cells responding to LPS with IL-1 secretion or a change in the binding affinity of the LPS receptor leading to a more active response.

Studies designed to resolve this question are in progress.

Monocytes have been shown to lose their potency as accessory cells after overnight culture (Mayernik *et al.*, 1983). This loss of potency has been attributed to the loss of surface HLA-DR expression by these cells (Unanue *et al.*, 1984). In this study, monocytes have also been shown to lose their ability to produce IL-1 after overnight culture (Table 4). Since both antigen presentation in the context of DR proteins and IL-1 production are important in the induction of human T lymphocyte activation (DeFreitas *et al.*, 1983), the decrease in both of these properties is probably responsible for the decreased potency of these cells in the induction of immune reactions. Previous reports have shown (Kelley, Fiers & Strom, 1984) and studies here confirm (Fig. 3), that IFN-γ is unique in its ability to maintain the level of expression of surface DR antigens on monocytes after overnight culture. This study points out that IFN-γ is also unique in its ability to maintain monocyte ability to produce IL-1 in response to stimulation with LPS. The role of IFN-γ in the preservation of monocyte IL-1 production complements the preservation of HLA-DR expression on these cells. Studies are under way to determine if this maintenance in ability to produce IL-1 is the result of preservation of responsiveness or enhancement of the residual responsiveness.

The ability of IFN-γ to influence IL-1 production in a positive fashion relates well with previous observations of the role of IFN-γ as an immune enhancing agent (Sonnenfeld & Merigan, 1979). This enhancement of IL-1 production provides a positive feedback cycle involving IL-1, IL-2 and IFN-γ production. All three events are interdependent with IFN-γ enhancement of IL-1 production leading to IL-1-mediated enhancement of IL-2 production leading to IL-2-mediated enhancement of IFN-γ production. This cycle appears to be negatively regulated by two major mechanisms. The first, and probably major, form of regulation is the requirement for the continued presence of an activating agent in all steps. Removal of the inducing agent, whether it be an antigen or mitogen, leads to a loss of production of all of these factors. Another agent which has been shown to negatively influence this cycle is prostaglandin E2 (PGE2). A number of studies have shown that monocytes in culture can produce PGE2 and that this molecule can inhibit the production of IL-2 and IFN-γ and the expression of macrophage Ia antigens (Walker *et al.*, 1983, Snyder, Beller & Unanue, 1982). Studies which demonstrate that PGE2 and IFN-γ are antagonists in their actions (Russell & Pace, 1984) reinforce this conclusion.

The recent clinical trials of IFN-γ indicate a pronounced pyrogenic activity associated with the purified protein (Sherwin *et al.*, 1984). These results of enhanced IL1 production in the presence of IFN-γ may suggest a potential mechanistic pathway to explain this property.

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