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Gamma Interferon Production by Combinations of Human Peripheral Blood Lymphocytes, Monocytes, and Cultured Macrophages

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Mitogen-induced interferon (IFN) production was studied using human peripheral blood mononuclear cells and subpopulations of lymphocytes, monocytes, and cultured macrophages. Cell populations were prepared in suspension to permit quantitative analysis of the interactions among different cell types. After stimulation by staphylococcal enterotoxin A, nylon column-purified lymphocytes produced only 5% as much IFN as the peripheral blood mononuclear cells from which they were prepared. When lymphocytes were supplemented with as little as 2% monocytes, IFN production increased two- to eightfold; with the addition of up to 20% monocytes, IFN production increased further, to levels approximating those of peripheral blood mononuclear cells. Monocytes alone produced no or very little IFN. Macrophages were derived from monocytes by culturing in vitro for 7 days. The addition of 2 to 5% autologous macrophages augmented IFN production to the same extent as 2 to 5% monocytes. However, more macrophages consistently resulted in less, rather than more, IFN, so that lymphocytes with 20% monocytes produced three- to eightfold more IFN than did lymphocytes with 20% macrophages. Thus, whereas the addition of monocytes over a broad dose-response range (2 to 20%) progressively augmented IFN production, macrophages showed an optimal effect at 2 to 5%, with higher percentages being inhibitory. The IFN induced by stimulation with staphylococcal enterotoxin A was characterized as IFN-γ by its resistance to neutralization by antibody to IFN-α and its inability to induce antiviral protection in embryonic bovine trachea cells.

Human peripheral blood mononuclear cells (PBMC) stimulated by mitogens or antigens produce "immune" interferon (IFN-γ) (6, 7, 16). Epstein et al. and Valle et al. in early studies of this response, observed that lymphocytes alone yield substantially less IFN-γ than do lymphocytes supplemented with cultured adherent macrophages (7, 22). Further investigations suggested that the cultured macrophages preferentially augment the production of IFN-γ (6), although IFN-γ can be obtained from freshly isolated human PBMC containing both lymphocytes and monocytes (3, 16, 22). We have reexamined the in vitro production of IFN-γ by improved cell preparation techniques which permit the quantitative titration of human peripheral blood lymphocytes with either freshly isolated monocytes or cultured macrophages.

MATERIALS AND METHODS

Preparation of PBMC populations. Human peripheral blood, in 10-ml portions, was diluted with an equal volume of Dulbecco phosphate-buffered saline without calcium and with 2 mmol of ethylene glycol-bis(β-aminoethyl ether)-*N*, *N*-tetra-acetic acid (pH 6.6), underlayered with 0.5 volume of Ficoll-Hypaque (Pharmacia, Inc., Piscataway, N.J.), and centrifuged at 400 × *g* for 30 min (1). The PBMC were harvested from the interface, washed with 40 ml of the phosphate-buffered saline solution and resuspended in 10 ml of the solution. The cells were then underlayered with 20 ml of 10% sucrose and 10 ml of 15% sucrose successively and centrifuged at 200 × *g* for 15 min at 4°C (2). The upper layers were discarded, and the pelleted cells were washed twice in unmodified phosphate-buffered saline. This procedure gave 1.5 × 10^6 to 2.1 × 10^6 PBMC per ml of whole blood (approximately 80% yield) with less than 1% contaminating erythrocytes or granulocytes. The preparations of PBMC were markedly depleted of platelets (2), which permitted the optimal recovery and culture of adherent monocytes.

Glass petri dishes (100 mm) were cleaned with detergent and treated with Siliclad (Clay Adams, Parsippany, N.J.) as detailed by Einstein et al. (5), plated with 50 × 10^6 PBMC in 5.5 ml of RPMI 1640 with 10% fresh autologous serum, and incubated for 2 h at 37°C in 5% CO_2. Nonadherent cells were recovered by washing the dishes three times with RPMI 1640. These nonadherent cells were further depleted of monocytes

and enriched for T lymphocytes by passing them over a nylon fiber column, as described by Greaves et al. (11). The resulting lymphocyte preparations represented 38 to 50% of the starting PBMC and contained less than 1% monocytes, as determined by peroxidase staining or latex particle phagocytosis.

Cells adhering to the glass dish were released by incubation with acetyl trypsin (40 U/ml; Sigma Chemical Co., St. Louis, Mo.) at 37°C for 15 min in 5 ml of Hanks balanced salt solution without Ca^{2+} or Mg^{2+} and with 0.60 mmol of EDTA. The reaction was stopped by the addition of 0.5 ml of 5% human serum albumin. Residual adherent cells were released by gentle scraping with a rubber policeman. The plate was then rinsed, and the cells were washed three times with Hanks balanced salt solution without Ca^{2+} or Mg^{2+}. These monocyte preparations represented 7 to 11% of the starting PBMC, or approximately 50% of the original monocytes. More than 90% of these cells were peroxidase positive, phagocytic, or both.

Macrophages were prepared by culturing glass-adherent cells in RPMI 1640 with 10% fresh autologous serum for 7 days; the medium was changed at day 3 or 4. As previously described, these cultured adherent cells undergo a pattern of morphological, biochemical, and functional changes consistent with cell differentiation (4, 5, 20). The macrophages were recovered by the same procedure used for the freshly adherent monocytes. Recovery procedures yielded from 4 to 8% of the PBMC originally plated, or more than 50% of the adherent monocytes; more than 98% of the cells were phagocytic and had the morphological appearance of macrophages.

Cell culture conditions. Cultures were performed in 16-mm plastic tissue culture wells (Linbro Co., New Haven, Conn.) in 1 ml of RPMI 1640 supplemented with 10% pooled AB serum, 25 μM 2-mercaptoethanol, 50 U of penicillin per ml, and 50 μg of streptomycin per ml. Cultures contained 1×10^6 PBMC or 1×10^6 lymphocytes plus 0 to 0.2×10^6 monocytes or macrophages. Mitogens used were phytohemagglutinin (PHA), 10 μg/ml (Phytohemagglutinin-P, Difco Laboratories, Detroit, Mich.), and staphylococcal enterotoxin A (SEA), 0.1 μg/ml (gift of Dr. Reginald W. Bennett, Food and Drug Administration, Washington, D.C.). These concentrations stimulated optimal IFN production. Cultures were harvested on day 3, unless otherwise indicated, and supernatants were stored at −70°C in Nunc vials (Vanguard Co., Neptune, N.J.) until assayed for IFN.

IFN assay. IFN in cell culture supernatants was assayed by a modification of the technique of Havell and Vilček (13), which we have previously described (18). Alterations in our method included the use of WISH cells (human cells; ATCC CCL 25) to assay for either IFN-α or IFN-γ (15, 16) and the use of embryonic bovine trachea (EBTr) cells (ATCC CCL 44), which are particularly sensitive to IFN-α (12). WISH cells were seeded 1 day before assay at 6×10^4 cells in 0.1 ml of Eagle minimum essential medium supplemented with vitamins, essential and nonessential amino acids, and 10% fetal bovine serum. EBTr cells were seeded 5 days before assay at 10^4 cells in Dulbecco modified eagle medium with 10% fetal bovine serum. Incubation of the cells with putative IFN challenge with vesicular stomatitis virus, and computation of the titer of antiviral activity were done as previously described (18). All IFN titers were expressed in international units on the basis of the titer of an IFN-α standard (Kaufman 73F 27/A; obtained from Dr. June Dunnick, Antivirals Substance Program of the National Institute of Allergy and Infectious Diseases) assayed in parallel.

IFNs were characterized by neutralization with rabbit anti-IFN-α (rabbit globulin 24 May 1974; obtained from J. Dunnick) by the method of Valle et al. (22). Combinations of 0.05 ml of IFN and 0.05 ml of antiserum were mixed in a microtiter plate in a checkerboard pattern with serial twofold dilutions of IFN (starting at 100 IU/ml) horizontally and serial twofold dilutions of antiserum (starting at 1:100 or 1:200) vertically. After incubation for 1 h at 37°C, the mixtures were removed and assayed for antiviral activity on WISH cells as described above. The titer of the antiserum against a sample of IFN was defined as the reciprocal of that dilution which neutralized 25 IU/ml (or 1.25 IU per well). The relative activity of the antiserum was calculated as 100 times the titer against the sample divided by the titer against the IFN-α standard.

RESULTS

Nylon column-passed lymphocyte preparations, which were depleted of monocytes, always produced less IFN than did the original mixed PBMC (Table 1). In experiments in which SEA was used as the mitogen, lymphocytes alone produced an average of only 5% as much IFN as did PBMC. When lymphocytes were supplemented with 20% monocytes, thus approximating the proportion of monocytes among PBMC, IFN production increased 5- to 40-fold, to levels at or slightly below that of PBMC. Purified lymphocytes stimulated with PHA also showed impaired IFN production, which increased when monocytes were added. In contrast to SEA, PHA-stimulated lymphocytes plus monocytes consistently yielded more IFN than did the unseparated PBMC. These different patterns of IFN production by PBMC compared with lymphocytes plus monocytes were observed in three consecutive experiments in which both mitogens were used, suggesting that they are characteristic responses of these cells populations to the different mitogens. Because SEA elicited more IFN than did PHA, the majority of our studies were performed with SEA, although all of the observations were confirmed with both mitogens. Unstimulated cultures of all cell combinations yielded no IFN in nine experiments.

When graded numbers of monocytes were added to the lymphocyte preparations, a clear titration effect was apparent (Fig. 1). Adding as little as 2 to 5% monocytes increased IFN production, but 10 to 20% supplements gave progressively more IFN. Although the sharpest percent increase in IFN production was observed with the initial increment of monocytes,

TABLE 1. IFN production by mitogen-stimulated PBMC compared with lymphocytes alone and lymphocytes supplemented with monocytes

Mitogen and expt	IFN (IU)		
	PBMC	Ly[a]	Ly + 20% Mono[b]
SEA			
1	600	10	400
2	2,400	75	1,600
3	3,200	150	800
4	7,757	484	7,757
5	7,757	242	2,900
6	3,200	400	4,687
PHA			
1	200	50	800
2	37	10	100
3	25	10	75
4	150	100	550
5	150	50	200

[a] Ly, Nylon column-purified lymphocytes.
[b] Mono, Freshly isolated glass-adherent monocytes.

appreciable increases were obtained with further increments.

In time course experiments, multiple replicate cultures were prepared on day 0, and individual cultures were harvested daily. Cultures of lymphocytes plus monocytes yielded more IFN than did lymphocytes alone on each day of culture (Fig. 2). For all cell combinations, IFN titers peaked on day 3 or 4. Stimulated monocytes alone (0.2×10^6/ml) yielded no IFN in four of five experiments and less than 3% as much IFN as yielded by unseparated PBMC in one experiment.

Glass-adherent monocytes held in culture for 7 days spread out and enlarged markedly as previously described (5, 7). These cells, which will be referred to as macrophages, were used to supplement mitogen-stimulated cultures of nylon column-passed lymphocytes. As with monocytes, macrophages plus autologous lymphocytes produced more IFN than did lymphocytes alone (Fig. 3 and Table 2). However, the quantitative titration of macrophages demonstrated marked differences between the ability of freshly isolated monocytes and of cultured macrophages to support IFN production. Whereas small numbers of macrophages (2 to 5%) consistently increased IFN production 3- to 10 fold, larger numbers (10 to 20%) decreased the yield of IFN. The relative effects of fresh monocytes and cultured macrophages were compared directly in a series of experiments in which both types of cells were added to the same preparation of autologous lymphocytes (Table 2). In six experiments, cultures of lymphocytes plus 2% (or 5%) macrophages or monocytes yielded almost identical titers of IFN. However, whereas the addition of 20% monocytes further increased IFN, cultures with 20% macrophages consistently yielded less IFN. In time course experiments, IFN appeared in comparable amounts over the first 2 days in cultures of PBMC and of lymphocytes with either 20% monocytes or 20% macrophages (Fig. 4). However, on days 3 and 4, IFN titers were appreciably depressed in cultures with 20% macrophages. The time course of IFN production by cultures of lymphocytes with either 2% monocytes or 2% macrophages was parallel to that of lymphocytes with 20% monocytes (data not shown). Stimulated cultures of macrophages alone (0.2×10^6/ml) yielded no IFN in four experiments.

The IFN produced by cultures of mitogen-stimulated PBMC cells or lymphocytes plus monocytes or macrophages was initially characterized as IFN-γ (immune IFN) by the lack of neutralization by antiserum against IFN-α ("leukocyte" IFN) (Table 3). Ten different samples of

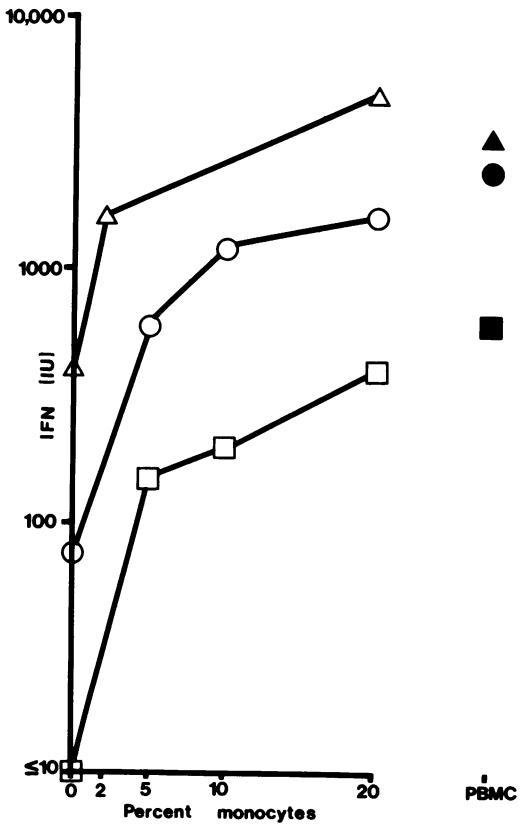

FIG. 1. SEA-stimulated IFN production by cultures of 10^6 nylon column-purified lymphocytes plus 0 to 20% monocytes (open symbols) and by the PBMC (closed symbols) from which the isolated subpopulations were prepared. The results of three independent experiments are shown.

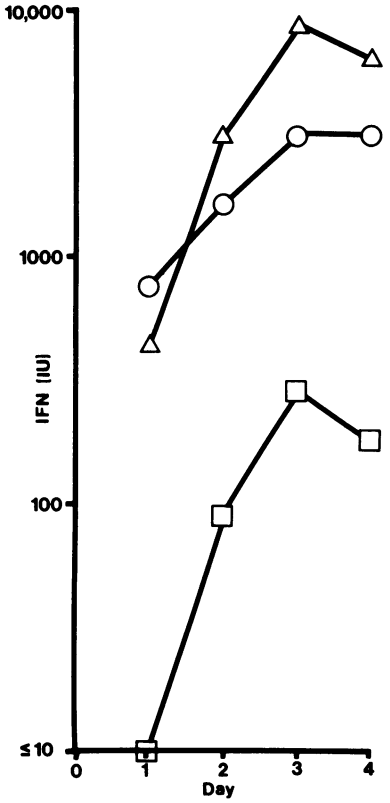

FIG. 2. Time course of SEA-stimulated IFN production by cultures of 10^6 nylon column-purified lymphocytes alone (□), by lymphocytes plus 20% monocytes (○), and by the PBMC from which the isolated subpopulations were prepared (△). Multiple replicate cultures were prepared on day 0, and individual cultures harvested on days 1 through 4.

mitogen-stimulated IFN, representing all cell combinations described, were analyzed using the checkerboard technique described by Valle et al. (22). The IFN-α standard at 25 IU/ml was neutralized by a 1:800 to 1:1,600 dilution of the antiserum in five separate experiments. A 1:100 dilution of antiserum failed to neutralize the antiviral effect of any sample of mitogen-induced IFN at any concentration, including 25 IU/ml. The antiserum to IFN-α thus had a relative activity of less than 10% against any mitogen-induced IFN.

The relative capacity to induce antiviral protection in EBTr cells is another method for differentiating between IFN-α and IFN-γ . The IFN-α standard had a 32-fold-higher titer of antiviral activity on EBTr cells than on WISH cells (Table 4) (12). In contrast, samples of mitogen-induced IFN which readily induced an antiviral effect on WISH cells failed to protect EBTr cells. When a mixture containing known titers of both types of IFN was assayed on EBTr cells, the expected titer of IFN-α was obtained, demonstrating that IFN-γ does not prevent the induction of antiviral protection (data not shown). Thus, IFN samples could be readily assayed on one cell line (WISH) and characterized by the use of another (EBTr).

DISCUSSION

Early studies by Epstein and co-workers and Valle et al. suggested that the production of mitogen-induced IFN requires an interaction between lymphocytes and adherent cells (6, 7, 22). Macrophage cover slips were prepared from peripheral blood monocytes cultured in vitro for 7 days. The addition of these macrophages to freshly isolated autologous lymphocytes resulted in a fivefold increase in IFN production after stimulation with PHA. In these experiments, the number of macrophages present was 2 to 10% of the number of lymphocytes

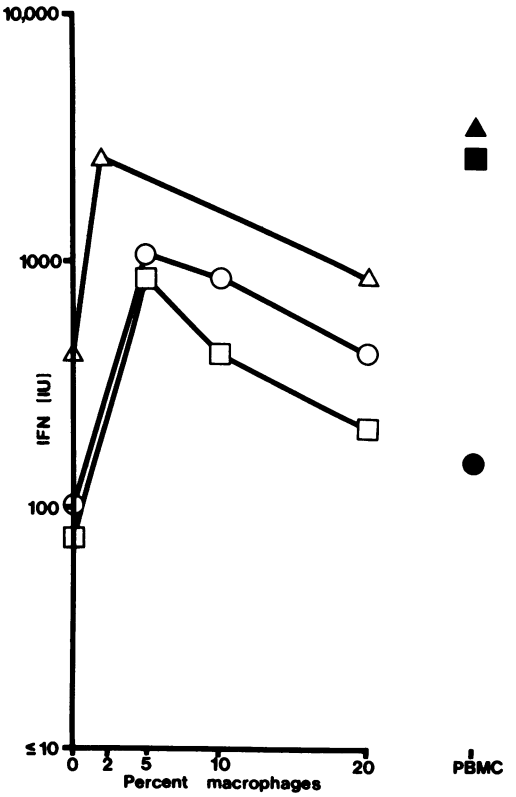

FIG. 3. IFN production by cultures of 10^6 nylon column-purified lymphocytes plus 0 to 20% autologous macrophages (open symbols) and by the PBMC (closed symbols) from which the lymphocytes were prepared. The results of three independent experiments are shown, two (△, ▲, □, ■) in which SEA was used and one (○, ●) in which PHA was used.

TABLE 2. Comparison of IFN production by lymphocytes alone and supplemented with monocytes or macrophages

Mitogen	IFN (IU)				
	Ly[a]	Ly + 2% Macro[b]	Ly + 2% Mono[c]	Ly + 20% Macro	Ly + 20% Mono
PHA (Expt 1)	50	100	100	75	200
SEA in expt:					
1	75	ND[d]	ND	200	1,400
2	150	300	400	200	800
3	484	1,939	1,939	1,454	7,757
4	400	2,400	1,600	800	4,687
5	400	1,600	2,262	1,130	3,200

[a] Ly, Nylon column-purified lymphocytes.
[b] Macro, Macrophages cultured for 7 days.
[c] Mono, Freshly isolated glass-adherent monocytes.
[d] ND, Not done. In this experiment, Ly + 5% Macro yielded 800 IU/ml, and Ly + 5% Mono yielded 600 IU/ml.

per culture. More recently, Goldblatt et al. have described an analogous system that uses macrophage cultures established in microwells to which lymphocytes are added directly (9). Our observations confirm these studies and further demonstrate that the addition of 2 to 5% macrophages is optimal for IFN production. Investigation of the dose-response pattern was possible because macrophages could be prepared in suspension in quantities sufficient for titration experiments. Furthermore, with both lymphocytes and macrophages (or monocytes) in suspension, experiments can be readily adapted to a microwell system (Arbeit and Levin, unpublished data). This is particularly desirable when cell numbers are limited, as in clinical studies.

Langford et al. have described the production of IFN-γ from PBMC stimulated with SEA (15–17). Our studies demonstrate that IFN induction by this mitogen also requires an interaction between lymphocytes and adherent cells, presumably circulating monocytes. Isolated subpopulations of lymphocytes or monocytes alone yielded little or no IFN, in contrast to cultures containing both cell types. The addition of monocytes over a wide dose-response range, at least 2 to 20%, reproducibly increased IFN production. Although small numbers (2 to 5%) of either monocytes and macrophages were equally effective, at higher percentages (up to 20%) only monocytes continued to augment IFN production.

Cultures of purified lymphocytes supplemented with 20% monocytes and stimulated with SEA yielded titers of IFN equal to or slightly less than the titers of IFN in cultures of unseparated PBMC (which contain approximately 20% monocytes). The manipulations required to isolate the specific cell subpopulations may have

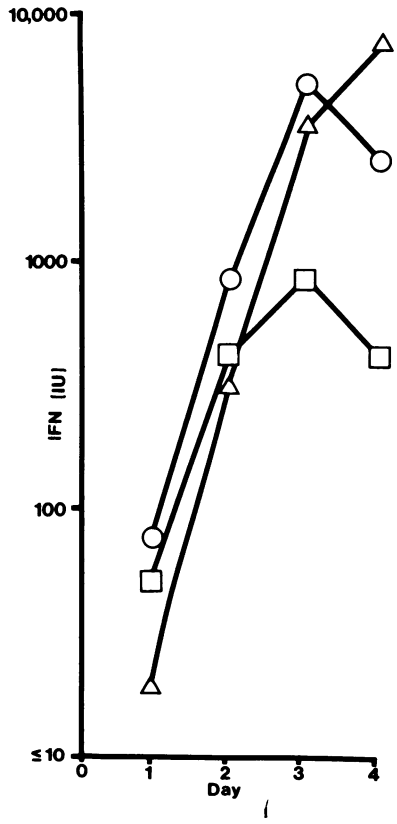

FIG. 4. Time course of SEA-stimulated IFN production by cultures of 10^6 nylon column-purified lymphocytes plus 20% monocytes (○) or 20% macrophages (□) and by the PBMC from which the lymphocytes and monocytes were prepared (△). Multiple replicate cultures were prepared on day 0, and individual cultures were harvested on days 1 through 4.

TABLE 3. Failure of antiserum against IFN-α to neutralize mitogen-induced IFN

IFN	Neutralization titer[a]	Relative activity[b] (%)
IFN-α standard	1,276[c] (800–1,600)	100
Mitogen-induced IFN sample (mitogen/cells)[d] in expt:		
1 SEA/PBMC	<100	<8
2 SEA/PBMC	<100	<8
3 PHA/PBMC	<100	<8
PHA/Ly + Mono	<100	<8
PHA/Ly + Macro	<100	<8
SEA/PBMC	<100	<8
SEA/Ly + Mono	<100	<8
SEA/Ly + Macro	<100	<8
4 SEA/PBMC	<200	<16
SEA/PBMC	<200	<16

[a] Neutralization titer is the dilution of anti-IFN-α antiserum which neutralized the IFN sample at 25 IU/ml.
[b] Relative activity = 100 × titer against sample/titer against standard.
[c] Geometric mean titer (range) for six experiments.
[d] Ly, Nylon column-purified lymphocytes; Mono, freshly isolated glass-adherent monocytes; Macro, macrophages cultured for 7 days.

slightly impaired their function. Alternatively, small subsets of cells required for optimal response may have been lost, for example, nylon-adherent lymphocytes or glass-nonadherent monocytes. In contrast, PHA-stimulated lymphocytes reconstituted with monocytes consistently produced more IFN than did the PBMC obtained directly from the Ficoll-Hypaque gradient. This observation suggests that in PBMC cultures stimulated by PHA there may be factors which suppress IFN production or shorten the half-life of IFN. Thus IFN-γ production in response to PHA, and perhaps other inducers, may be further modulated by additional cell subpopulations found among PBMC but not included in the separated lymphocytes and monocytes used in these studies.

Many parameters of the immune response to antigens and mitogens, including lymphocyte proliferation, lymphokine production, and immunoglobulin synthesis, require interaction between lymphocytes and accessory adherent

TABLE 4. Comparison of the absolute titers of IFN-α and IFN-γ assayed on WISH cells and EBTr cells

IFN	Titer	
	WISH	EBTr
IFN-α[a]	41[b] (16–128)	1,395[b] (256–2,048)
IFN-γ pool[c]	676[d] (256–2,048)	<2[d] (all < 2)
Mitogen-induced IFN-γ sample (mitogen/cells)[e]		
PHA/PBMC	64	<2
PHA/Ly + Mono	128	<2
PHA/Ly + Macro	256	<2
SEA/PBMC	1,024	<2
SEA/Ly + Mono	1,024	<2
SEA/Ly + Macro	64	<2
SEA/PBMC	1,024	<2
SEA/PBMC	1,024	<2

[a] IFN-α standard at 100 IU/ml.
[b] Geometric mean titer (range) for eight experiments.
[c] Internal laboratory standard of IFN pool from SEA-stimulated PBMC.
[d] Geometric mean titer (range) for six experiments.
[e] See Table 3, footnote *d*.

cells. Our observations demonstrate a similar requirement for the production of IFN-γ. Mitogen-induced lymphocyte proliferation is, in general, least impaired by the depletion of adherent cells with the usual techniques. In our experiments, stimulated lymphocytes alone incorporated approximately 50% as much [^{3}H]thymidine as did unseparated PBMC. Proliferation was essentially fully restored by the addition of 2% monocytes, with little, if any, further increase with up to 20% monocytes (data not shown). Thus, IFN-γ production was more sensitive to monocyte depletion and supplementation than to lymphocyte proliferation, and these two parameters did not closely correlate in cultures of lymphocytes plus monocytes.

Macrophage suppression of immune function has been described under various experimental conditions and in various diseases (10, 20, 23). Cultured macrophages derived from normal circulating monocytes by techniques similar to ours have been reported to suppress mitogen-induced lymphocyte proliferation (20). In our experiments, lymphocyte cultures supplemented with 2 to 5% macrophages showed a small, but consistent, increase in [^{3}H]thymidine incorporation compared with lymphocytes alone. However, in cultures of lymphocytes plus 20% macrophages, proliferation was generally suppressed by more than 50%. Cell viabilities were similar in cultures with monocytes or macrophages, but the percentage of lymphoblasts was decreased in cultures with macrophages (data not shown). Thus, the biphasic effect of progressively increasing numbers of macrophages on lymphocyte cultures was observed for both IFN production and lymphocyte proliferation. Although the mechanism and significance of this pattern has not been denied, it suggests that increased numbers of macrophages may suppress many different parameters of the immune response, including lymphocyte proliferation and IFN production. Alternatively, the cultured macrophages, which have been shown to possess enzymes different from those of circulating monocytes (4), may be inactivating the IFN produced.

Although these studies demonstrate the interaction between lymphocytes and adherent cells in the production of IFN-γ, there is no direct evidence to establish which cell type is actually making the IFN. IFN-γ has been generally regarded as a lymphokine, i.e., a product of an activated lymphocyte. Epstein et al. observed that cotton column-purified lymphocytes alone usually made some IFN, whereas cultured macrophages alone did not (7). However, using techniques which permit the quantitative titration of monocytes or macrophages and lymphocytes, we have observed that as little as 2% adherent cells will markedly increase IFN production by nylon column-purified lymphocytes. Since adherence column separation techniques alone do not reliably deplete adherent cells below the level of approximately 1%, it is possible that the few monocytes contaminating the lymphocyte preparations are responsible for the small, variable titers of IFN produced by cultures of lymphocytes alone. Isolated monocytes, which contained small numbers of contaminating lymphocytes, occasionally gave low titers of IFN. Macrophage preparations, which after 7 days in culture contained essentially no lymphocytes, never yielded IFN. Thus, adherent cells alone also are probably not sufficient to yield IFN. Adherent cells may be required to support the appropriate activation of lymphocytes to produce IFN-γ. Alternatively, mitogen-activated lymphocytes may in turn stimulate adherent cells to produce IFN-γ; i.e., it is a monokine, the product of activated monocytes. Studies with murine splenocytes have also consistently demonstrated that the production of IFN-γ requires the interaction between lymphocytes and macrophages, but there are conflicting reports defining which cell type is actually producing the IFN (8, 14, 19, 21).

Careful characterization of the type of IFN elicited from human PBMC by particular stimuli is necessary for understanding the physiological significance of the response. Initially, physicochemical properties, including lability to heat and acid treatment, were used to distinguish IFN-γ from IFN-α (6, 22). In our experiments, mitogen-induced IFN was consistently inactivated after dialysis at pH 2, whereas the IFN-α standard was stable (data not shown). However, the dialysis and recovery of multiple small samples were technically tedious. Heat lability was too variable to be reliable. Antiserum to IFN-α, which does not inactivate IFN-γ, is extremely useful in characterization studies. However, the quantitative use of the antiserum, as described by Valle and co-workers, requires a separate microtiter plate for each sample analyzed and uses appreciable amounts of this still scarce reagent (22). EBTr cells provide a simple and highly sensitive method for characterizing IFN samples. Although the absolute titer of IFN-α is approximately 32-fold greater on EBTr cells than on WISH cells (12), samples of mitogen-induced IFN with titers of up to 1,024 on WISH cells were undetectable on EBTr cells. It can therefore be calculated that the samples of mitogen-induced IFN contained less than 1 IU of IFN-α per ml. EBTr cells are particularly useful in quantitatively analyzing specimens containing both types of IFN, such as the supernatants of cultures of PBMC stimulated by specific viral antigens (M. J. Levin, R. D. Arbeit, and P. L.

Leary, manuscript in preparation). However, the observation that different types of IFNs have divergent activities on different cell lines underscores the problems of standardizing a bioassay and highlights the need for an IFN-γ reference standard analogous to that now available for IFN-α.

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