INTERFERON INHIBITS THE GENERATION OF ALLOSPECIFIC SUPPRESSOR T LYMPHOCYTES

By DIDIER FRADELIZI and ION GRESSER

From the Laboratoire des Interactions Lymphocytaires, Centre Hayem, Hôpital Saint-Louis, 75475 Paris Cedex 10, France; and the Laboratorie d'Oncologie virale, Institut de Recherches Scientifiques sur le Cancer, BP 8,94802 VILLEJUIF Cedex, France

Mouse and human interferon can modify the immune response in different and seemingly contradictory ways (1). Thus, interferon preparations inhibit lymphocyte proliferation in a primary mixed lymphocyte reaction (MLR)[1] (2–5) but, on the other hand, enhance lymphocytotoxicity (3–6). We wanted to determine the action of interferon on the generation of other functional lymphocyte subsets, such as allospecific primed lymphocytes (7, 8) or suppressor T lymphocytes (9–12).

The results presented herein show that human interferon inhibits the generation of allospecific suppressor T lymphocytes that normally develop from lymphocytes primed in vitro against allogeneic cells (9–12). This effect might influence considerably the generation of other functional lymphocyte subsets during an allogeneic response, such as cytotoxic effector cells and allospecific primed lymphocytes.

Materials and Methods

Donors. Normal individuals and families were HLA-A, HLA-B, and HLA-C typed with highly selected local and VIIIth Workshop typing sera, with the standard National Institutes of Health two-stage complement-dependent lymphocytotoxicity technique at room temperature. B lymphocyte DRw determinants were typed on B-enriched cell suspensions with an adaptation of the same technique (13).

Medium. Tissue culture medium was RPMI 1640 (Gibco Laboratories, Grand Island Biological Co., Grand Island, NY) buffered with $NaHCO_3$, supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), and fungizone (250 μg/ml), and enriched with normal human serum.

Lymphocytes. Peripheral lymphocytes were purified from heparinized blood on a Ficoll-Isopaque gradient (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, NJ) (δ = 1.078 g/cm^3). Cells were either used immediately or frozen and stored in liquid nitrogen (10).

MLR. A micro technique routinely used in this laboratory was used. Briefly, fresh or thawed peripheral blood lymphocytes were cultured in round-bottomed microtiter plates (Greiner, Nürtigen, Federal Republic of Germany) in triplicate. To each well was added 0.5 × 10^5 responder cells in 0.1 ml of TCM and 0.5 × 10^5 stimulating cells in 0.05 ml. The stimulating cells were irradiated with 2,500 rad from a ^{60}Co source (gamma cell irradiator, Atomic Energy of Canada, Ottawa). TCM was supplemented with 20% normal human serum. Various doses of interferon were added in 0.05-ml volumes to each well. Plates were incubated at 37°C in a humidified 5% CO_2 air atmosphere. After 96 h, the cultures were pulse-labeled with [^{3}H]thymidine (1 μCi/well) (spec act 5 Ci/mmol; Commissariat à l'Energie Atomique, Saclay, France). 18 h later, cultured cells were harvested with a multiple sample precipitator (Otto Hiller, Madison) on glass fiber filters (Watman). Filters were dried, placed in 3 ml PPO-

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[1] *Abbreviations used in this paper:* MLR, mixed lymphocyte reaction; PHA, phytohemagglutinin.

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POPOP, and counted in an Intertechnique (SL30) liquid scintillation counter. The median value of triplicate cultures was used for data analysis.

Cell-mediated Lympholysis. Lymphocytes sensitized in vitro for 5 or 6 d against allogeneic stimulating cells in the presence or absence of various doses of interferon were washed and used as effectors against phytohemagglutinin (PHA)-treated ^{51}Cr-labeled target lymphocytes. Usually, 10×10^6 target cells were labeled with 250 μCi of ^{51}Cr in 10 ml of RPMI 1640 for 18 h. The cytotoxic assay was performed in round-bottomed microtiter plates (Linbro Chemical Co., Hamden, CT) with 1×10^4 target cells per well and different effector to target cell ratios: 50:1, 25:1, 10:1, and 5:1. After an initial centrifugation of 2 min, microplates were incubated at 37°C for 4 h and then centrifuged for 10 min. The supernatants were collected with a semiautomatic apparatus (Skraton Titertek; Flow Laboratories, Inc., Rockville, MD), and radioactivity was counted in an Intertechnique γ-scintillation counter. Spontaneous ^{51}Cr release was determined from target cells incubated in media alone, and maximum release was determined from cells treated with 1 N HCl. Specific release was calculated as follows:

$$\text{specific release} = ([\text{cpm experimental} - \text{cpm spontaneous}]/[\text{cpm maximal} - \text{cpm spontaneous}]) \times 100$$

In these studies, a lytic unit was defined as the number of cytotoxic T lymphocyte required to cause 20% killing, as measured by ^{51}Cr release (14, 15).

Primed Lymphocyte Typing. For primary cultures, 10×10^6 lymphocytes were sensitized in vitro in the presence of various doses of interferon by incubating with 10×10^6 (2,500 rad irradiated) stimulating cells in 20 ml TCM, supplemented with 10% human serum at 37°C in a humidified 5% CO_2 air atmosphere (16, 17). After 10–11 d, cells were harvested and carefully washed before secondary cultures were initiated.

For secondary cultures, the sensitized responder cells were adjusted to 0.1×10^6 viable cells per ml in TCM, 20% human plasma and restimulated with fresh or thawed stimulating cells. Cells were distributed in V-bottomed plastic culture trays (Microtiter, Greiner). 1×10^4 responder cells in 0.1 ml volume and 5×10^4 stimulator cells in 0.05 ml were added to each well. After 48–72 h of incubation, cultures were pulse-labeled for 6 h using [^{3}H]thymidine (1 μCi/well) (spec act 5 Ci/mmol), and incorporated radioactivity was counted in a liquid scintillation counter (16, 17).

Evaluation of Suppression with Suppressor Cells. Suppressor activity of in vitro sensitized lymphocytes (primed once or twice) was evaluated with a three-cell assay. This three-cell assay is a modification of the classical MLR microtechnique (10). Briefly, fresh or thawed lymphocytes were cultured in round-bottomed microtiter plates (Greiner) in triplicate in TCM. The three co-cultured cells mixed in each well were (*a*) responder cells (5×10^4 in 0.1 ml), (*b*) irradiated (2,500 rad) stimulating cells (5×10^4 in 0.05 ml), and (*c*) an irradiated (2,500 rad) third-cell population of either primed cells (possible suppressor cells) or unprimed control cells (5×10^4 in 0.05 ml TCM). Plates were incubated at 37°C in a humidified 5% CO_2 atmosphere. After 96 h, the cultures were pulse labeled with [^{3}H]thymidine for 18 h, and incorporated radioactivity was counted. The median value of triplicate cultures was used for data analysis. The percentage of suppression was calculated with the following formula:

$$1 - ([\text{test cpm median value}]/[\text{control cpm median value}]) \times 100$$

Interferon Preparations. Human interferon was prepared and purified by K. Mogensen, as previously described (18, 19). The partially purified and highly purified interferons had specific activities of 10^6 U/mg protein and 10^8 U/mg protein, respectively. Contaminating proteins were recovered from the purification and used as mock interferon.

Results

Effect of Human Interferon on the Primary MLR. The effect of various doses of human interferon on three parameters of the MLR was studied: proliferation of lymphocytes, recovery of primed lymphocytes, and generation of cytotoxic lymphocytes.

Proliferative Response. A series of experiments showed that 10^3 to 10^4 U/ml of

human interferon α consistently inhibited the proliferative response on days 6 or 7 culture, as determined by [^{3}H]thymidine incorporation, whereas only a slight delay in the response was observed in cultures incubated with 10^1 to 10^2 U/ml of interferon (day 6) (Fig. 1). Such dose-dependent inhibition of the proliferation in MLR was observed in every experiment, although the extent of inhibition varied in different experiments.

Recovery of Lymphocytes in MLR and Cytotoxicity of These Lymphocytes. As can be seen in the legend to Fig. 1, the recovery of primed lymphocytes on day 11 of culture varied between 150 and 240% in cultures incubated with interferon doses ranging from 10^0 to 10^2 U/ml, and 100% and 40% from cultures incubated with 10^3 and 10^4 U/ml.

The cytotoxicity of lymphocytes collected on day 6 of the MLR was tested in a 4-h chromium release assay using PHA-stimulated lymphoblasts as target cells. The number of cytotoxicity effector cells was evaluated using different effector to target cell ratios and calculating the lytic units per culture (see Methods and Materials). As

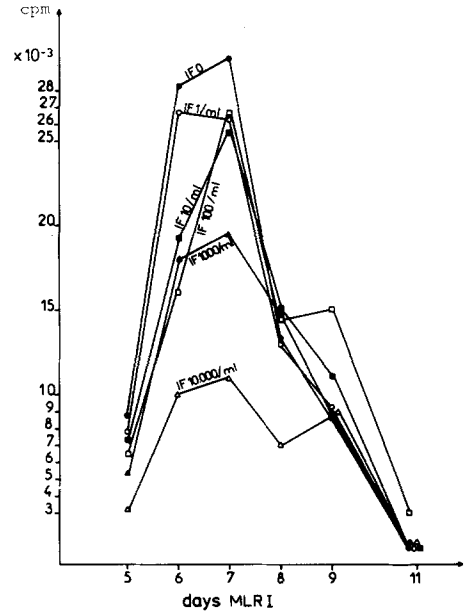

FIG. 1. Kinetic of the primary proliferative response of human lymphocytes responding to 2,500 rad irradiated stimulating cells in the presence of various doses of interferon. In this experiment the number of viable lymphocytes recovered per 0.25 × 10^6 cells cultured initially was as follows:

Interferon	Day 7		Day 11	
	× 10^6	Percent recovery of culture	× 10^6/ml	Percent recovery of culture
U/ml				
0	0,68	272%	0,5	200%
10^0	0,63	276%	0,5	200%
10^1	0,68	272%	0,6	240%
10^2	0,50	200%	0,38	152%
10^3	0,40	160%	0,25	100%
10^4	0,23	92%	0,10	40%

can be seen from the two experiments summarized in Table I, addition of 10^1 or 10^2 U/ml of interferon α in the primary MLR resulted in a two- to sixfold increase in cytotoxicity. The increase was less pronounced for cultures incubated with 10^3 U/ml of interferon, which might be related to the inhibitory effect on lymphocyte proliferation.

Effect of Interferon on the Generation of Memory-primed Cells That Exhibit an Accelerated Secondary Proliferative Response. After in vitro allogeneic sensitization, specific memory-primed lymphocytes exhibit an accelerated proliferative response when restimulated by the specific cells used in the primary MLR (7, 8). Memory-primed cells recognize mainly HLA-DR antigens, although the specificity of recognition of HLA-DR antigen (discriminative ability) is variable (16).

It was of interest to determine, therefore, the effect of interferon in the primary MLR on (*a*) the generation of memory cells and (*b*) the capacity of the primed lymphocytes to show specific allogeneic reactivity for HLA-DR antigens. Accordingly, in five experiments, the proliferation of lymphocytes on day 7 of primary allogeneic sensitization was evaluated in the presence of various doses of interferon α, and the specific secondary proliferative response of these lymphocytes recovered from cultures on day 10–11 was evaluated without interferon. As can be seen from Fig. 2, lymphocytes primed in the presence of 10^0 to 10^2 units of interferon exhibited a normal or slightly increased specific secondary proliferation.

To test the effect of interferon on the specificity of primed lymphocytes, we used responding and stimulating lymphocytes from family members differing for only one HLA haplotype. These lymphocytes were then tested for their accelerated secondary proliferative response against a panel of stimulating cells. In one typical experiment, illustrated in Table II, lymphocytes from donor E (HLA-DR2/DR-) were primed for

TABLE I

Effect of Interferon on the Generation of Cytotoxic Cells during MLR

Experiment	Interferon	Target cells		
		Autologous	Specific target cell	Cells of HLA identical sib-specific target cell donors
	U/ml			
1	0	0*	9*	4, 5*
	1	0	12	6
	10	0	20	14
	100	0	21	16
2	0	0	11	33
	10	0	68	180
	100	0	66	222
	1,000	0	33	40

Mixed lymphocyte reactions were performed in the presence of various doses of interferon. Lymphocytes recovered on day 6 from cultures were washed and tested for cytotoxicity. Cytotoxicity was evaluated by a 4-h chromium release assay using 10^4 PHA-induced blasts as target cells. 50/1, 25/1, and 10/1 effector to target cell ratios were used. Results are expressed in lytic units per culture.

* Lytic units per 10^6 recovered lymphocytes.

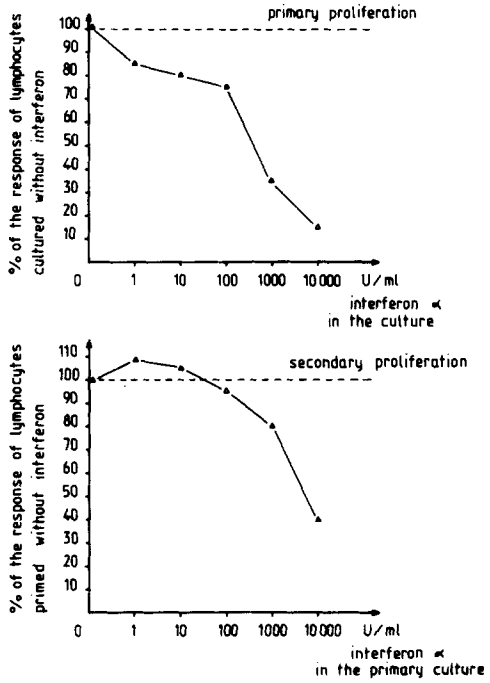

FIG. 2. Day 7 proliferation of lymphocytes sensitized in the presence of interferon α (a) and secondary specific response (without interferon) of primed cells collected from these cultures (b). Δ, percent response calculated from median cpm value of triplicate cultures.

10 d against irradiated cells from his haplo-identical sibling G (HLA-DR2/DR3). These lymphocytes, primed against HLA-DR3, showed an accelerated proliferative response when incubated with lymphocytes exhibiting DR3 antigens and almost as high a response when incubated with lymphocytes from DR3-negative individuals (DR7/DR2 or DR5/DR2). This nonspecific typing response is occasionally observed with in vitro primed cells (16). However, when primed lymphocytes recovered from cultures incubated with interferon were tested (Table II), it is evident that a good specific secondary response was observed against lymphocytes from DR3-positive donors (regardless of the dose of interferon, 10^1 to 10^3 U/ml). In addition, with increasing amounts of interferon in the primary MLR, the extent of the nonspecific response (i.e., against lymphocytes from DR3-negative donors) was considerably reduced. Thus, primed cells generated in an MLR in the presence of interferon showed a greater specificity in the secondary allogeneic response than those cultured without interferon.

We conclude from these experiments that interferon inhibits the proliferative response in the primary MLR and enhances the generation of cytotoxic lymphocytes. Despite the inhibition of lymphocyte proliferation in the MLR, low doses of interferon (<1,000 U/ml) do not significantly affect the generation of memory-primed lymphocytes. Furthermore, interferon seemed to induce a specific enhanced discriminatory capacity of these memory-primed cells.

Effect of Interferon on the Generation of Allospecific Suppressor T Lymphocytes. Allospecific suppressor T lymphocytes are generated in the MLR after one or several stimulations

TABLE II

Effect of Interferon on the Specific Recognition of HLA-DR Antigens by In Vitro Primed Lymphocytes

Stimulating cells (family members)	Secondary allogeneic response of anti-HLA-DR3 primed lymphocytes after the sensitization in culture (primary MLR)			
	Interferon in primary MLR			
	No interferon	10 U/ml	100 U/ml	1,000 U/ml
HLA-DR antigens				
DR3/DR2	27,100*	25,800	30,800	34,100
$DR3^+$				
DR3/DR7	29,500	27,200	26,000	28,900
DR2/DR3	25,800	23,600	25,800	26,800
Specific control				
$DR3^-$				
DR7/DR2	23,100	10,600	14,300	10,500
DR 5/DR2	18,500	15,800	8,800	9,400
DR 2/DR^-	3,000	2,000	3,100	2,000
Negative control				

* Median cpm value of triplicate cultures. [^{3}H]Thymidine incorporation for the last 6 h of culture.

Lymphocytes from individual E (HLA DR2/-) were sensitized in vitro against irradiated stimulating cells from his haplo-identical sib G (HLA DR2/DR3). Sensitization cultures were incubated with various doses of interferon. Anti-HLA DR3-primed E lymphocytes were collected on day 11, washed, and tested for their secondary response against stimulating lymphocytes of various family members. Proliferation was evaluated after 72 h of secondary cultures.

with allogeneic cells (10–12). These suppressor cells are radioresistant and can inhibit the proliferative response of fresh unprimed autologous lymphocytes, stimulated by cells possessing the HLA-DR antigens used initially for priming. Suppressor activity can be evaluated in a "three cell assay" in which the following three types of lymphocyte are cultured: (*a*) responding lymphocytes: these are fresh unprimed cells autologous to the primed lymphocytes; (*b*) irradiated allogeneic stimulating cells; (*c*) primed cells recovered from the initial MLR. These cells are x-irradiated. If they exert suppressor activity, they will inhibit the response of (*a*) stimulated by (*b*). In control cultures these cells (*c*) are replaced by fresh unprimed irradiated autologous cells.

Experiments were undertaken to determine the generation of suppressor T lymphocytes after allogeneic sensitization in the presence of interferon. Primed lymphocytes recovered from a 10-d or 11-d MLR were washed, irradiated, and tested for suppressor activity in a three-cell assay. Lymphocytes from members of HLA genotyped families were used. The priming combinations were between haplo-identical (one haplotype shared) individuals to generate suppressor T lymphocytes specific for only one HLA-DR antigen.

Lymphocytes from individual J (HLA-DR 8/7) were primed in vitro against cells from his haplo-identical brother C (HLA-DR 8/3) without interferon or with varying amounts of interferon (Table III). Primed lymphocytes, J anti-C (anti-DR3), were collected on day 11, washed, irradiated, and tested in a three-cell assay in which fresh,

TABLE III

Specific Suppressor T Lymphocytes Tested in a Three-Cell Assay

Responding lymphocytes	Stimulating cells*	Third cells added									
		Control cultures			Test cultures, primed J lymphocytes* (anti-HLA-DR 3)						
		Unprimed J lymphocytes* proliferation control	With no interferon suppressor control		1 U/ml interferon		10 U/ml interferon		100 U/ml interferon		1,000 U/ml interferon	
		cpm‡	*cpm*	*% suppression*	*cpm*	*% suppression*	*cpm*	*% suppression*	*cpm*	*% suppression*	*cpm*	*% suppression*
J lymphocytes unprimed (DR8/7)	HLA DR family members											
	F = DR 8/11	17,400	16,000	6	22,000	0	27,000	0	17,300	0	18,200	0
	M = DR 7/3	27,000	1,000	96	1,700	93	9,900	63	22,000	18	29,300	0
	B = DR 8/3	28,000	3,000	90	7,000	75	21,000	25	27,000	4	25,000	10
	O = DR 8/3 C = DR 8/3	28,000	2,800	90	5,600	80	19,000	29	30,000	0	24,500	12
	Specific control J = DR 8/7	57,100	3,900	93	2,700	95	28,500	50	48,100	16	44,200	22
	Negative control	700	300		1,000		1,000		600		500	
	Unrelated individual											
	DR 2/7	23,100	27,300	0	21,500	7	25,800	0	ND§		24,600	0
	DR 5/3	41,900	1,700	96	8,400	80	32,000	23	ND		45,500	0

* 2,500 rad-irradiated cells.

‡ Median cpm value of triplicate cultures. [^{3}H]Thymidine incorporation on day 6 of mixed culture.

Lymphocytes from individual J (HLA DR 8/7) were primed against cells from his haplo-identical brother C* (HLA DR 8/3) in the presence of various doses of interferon. Primed lymphocyte J anti-C* (anti-HLA DR3) were collected on day 11, washed, and tested for suppressor activity in a three-cell assay in which fresh unprimed J lymphocytes were stimulated by irradiated cells from members of the family and from additional individuals possessing or not possessing the HLA DR3 antigen used for priming. Proliferation was evaluated on day 6 of MLC.

unprimed J lymphocytes were stimulated by lymphocytes of members of the family and of two unrelated individuals, one bearing the specific DR antigen used for priming (i.e., DR3) or one with irrelevant antigens (DR 2/7). As can be seen in Table III, J primed lymphocytes exerted a specific suppressor effect when incubated in a three cell assay with lymphocytes from individual C (DR 8/3) (i.e., compare 57,100 cpm in control cultures to 3,900 cpm, 93% suppression), with lymphocytes from family members M, B, O, bearing DR/3, or with cells from an unrelated DR 5/3 individual. No suppression was observed when J primed lymphocytes or unprimed lymphocytes were incubated with family member F (DR 8/11) or unrelated individual (DR 2/7), whose lymphocytes did not carry DR-3. Thus, the suppressor activity of J primed lymphocytes was specific and was only manifest when tested in the presence of lymphocytes bearing the same surface antigen (DR-3) that had been used for sensitization. When J lymphocytes had been primed in the presence of 10 U/ml or more of interferon, the extent of specific suppression exerted in the three-cell assay decreased with increasing amounts of interferon. For example, when J lymphocytes primed in the presence of 10^3 U/ml of interferon were tested in a three-cell assay (with fresh J lymphocytes) with lymphocytes of individuals M, B, O, and C, slight or no suppression was observed, (e.g., for individual C, compare 3,900 cpm, 93% suppression, with 44,200 cpm, 22% suppression; for individual M, primed lymphocytes in the absence of interferon showed 96% suppression, whereas lymphocytes primed with 10^3 U/ml of interferon showed no suppression.

Three other experiments were undertaken using cells from various HLA-typed donors from other families, and in each instance lymphocytes primed in the presence of 10^2 or 10^3 U/ml of interferon no longer exerted a suppressor effect on the proliferative response of fresh stimulated lymphocytes. These experiments were performed using highly purified interferon α (spec act 10^8 U/mg protein). The impurities collected in the course of purification did not inhibit the generation of suppressor cells.

Studies to Elucidate the Mechanism(s) by Which Interferon Inhibits the Generation of Allosuppressor T Lymphocytes. Although several hypotheses might be proposed to explain how interferon inhibited the generation of specific allosuppressor T lymphocytes, we postulated that interferon inhibited the development of presuppressor to functional suppressor lymphocytes. Fig. 3 illustrates the experimental protocol we used to test this hypothesis. As discussed above (section III), J lymphocytes (HLA-DR8/7) primed by C lymphocytes (HLA-DR 8/3), in the presence of 100 U/ml of highly purified interferon α, exhibited a marked reduction in suppressive activity in the three-cell assay (Table III). In the following set of experiments, J lymphocytes, primed in the presence of interferon (and lacking suppressor activity), were washed thoroughly to remove interferon and stimulated in a secondary culture with C lymphocytes (HLA-DR 8/3) in the absence of interferon (Fig. 3). After 7 d of secondary culture, the twice-primed lymphocytes (anti-DR3) were tested for their suppressor activity in a three-cell assay. As can be seen in Table IV, J lymphocytes, which showed only minimal suppressor activity when initially primed in the presence of interferon, did exert specific suppressor activity on fresh autologous lymphocytes after secondary stimulation by DR-3-positive cells. The suppression was specific for cells bearing DR-3 antigen, because suppression was not observed when F cells (HLA-DR 8/11) were added to the three-cell assay. We conclude from these experiments that interferon inhibits the generation of specific suppressor cells but does not destroy these cells or their precursors, because subsequent stimulation of these cells by the same stimulating cells in the absence of interferon results in full antigen-specific suppressor activity.

Additional experiments were performed to determine whether lymphocytes primed in the presence of interferon and failing to exert suppressor activity could, nevertheless, develop antigen-specific suppressor activity upon secondary stimulation in the presence of 100 U/ml interferon. The results (not shown) indicate that suppressor

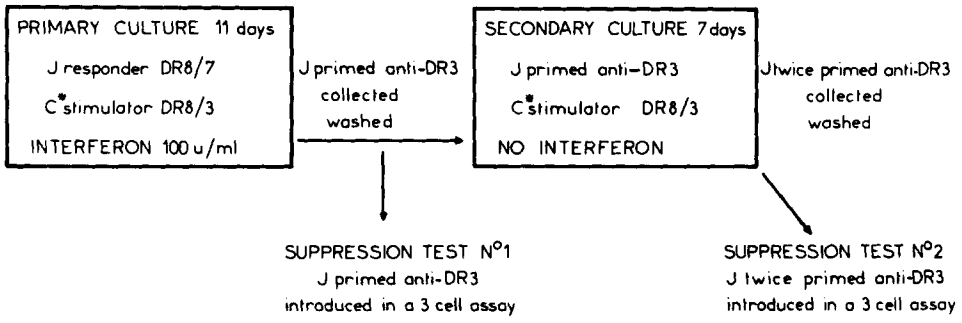

FIG. 3. Generation of suppressor T lymphocytes in the presence of interferon. Effect of two successive allogenic stimulations. Asterisk indicates irradiated stimulating cells.

TABLE IV

Effect of Interferon α on the Generation of Suppressor T Lymphocytes. Effect of Two Successive Allogeneic Stimulations

Responding cells	Stimulating cells (irradiated)	Third cell added suppression test number 1					Suppression test number 2				
		Control cultures J cells unprimed	J primed once against C* (anti-DR3) in primary culture				Control cultures J cells unprimed	Test cultures, J primed twice against C* (anti-DR3)			
		Proliferation control	No interferon suppression control		100 U/ml interferon in the primary culture		Proliferation control	No interferon suppression control		100 U/ml interferon in the primary culture (no interferon in the secondary culture)	
		*cpm**	*cpm*	*% suppression‡*	*cpm*	*% suppression*	*cpm*	*cpm*	*% suppression*	*cpm*	*% suppression*
J lymphocytes unprimed	HLA DR family members										
	F = DR 8/4	17,400	16,000	6	17,300	0	17,900	15,500	13	16,600	7
	M = DR 7/3	27,000	1,000	96	22,000	18	20,500	500	97	900	95
	C = DR 8/3										
	Specific control	57,100	3,900	93	48,100	16	18,800	300	98	500	97
	J = DR 8/7										
	Negative control	700	300		600		500	800		300	

* Median cpm value of triplicate cultures. [^{3}H]Thymidine incorporation on day 6 of mixed lymphocyte cultures.

‡ Percent suppression calculated using the formula: percent suppression = 1 − ([cpm test in suppressed culture]/[cpm in proliferation control]) × 100. Lymphocytes from individual J (HLA-DR 8/7) were primed in vitro against cells from his haplo-identical brother C* (HLA DR 8/3) in the presence of 100 U/ml interferon (same cells as in Table III). Primed J lymphocyte anti-C* (anti-HLA-DR3) were collected on day 11, washed, and either tested immediately for suppressor activity in a three-cell assay or stimulated a second time with the specific C* cells for 7 d without interferon. J lymphocytes primed twice against C* were then tested for their suppressive activity and compared to J primed only once against C*. Proliferation was evaluated on day 6 of MLC.

lymphocytes can develop full antigen-specific suppressor activity after secondary stimulation even in the presence of interferon. We conclude that interferon inhibits the generation of specific suppressor cell during the first allogeneic stimulation but not thereafter.

Discussion

The most striking results in these studies concerned the inhibitory effect of interferon on the generation of suppressor lymphocytes capable of suppressing the proliferative response of autologous lymphocytes to allogeneic cells. Previous studies in mice (12) and in man have shown that allospecific suppressor T lymphocytes are generated in vivo (9) and in vitro (10, 11) after one or several stimulations with allogeneic cells. These suppressor cells are radioresistant and specific for the HLA-DR antigen presented by the cells initially used for priming. Lymphocytes primed in vitro in the presence of $\geq$10 U/ml of interferon showed a marked decrease in suppressor activity (Table III). To determine the mechanism by which interferon inhibited suppressor activity, we restimulated primed interferon-treated lymphocytes (which showed no suppressor activity). The results of these experiments showed that restimulation of these cells did result in the generation of suppressor cells whether interferon was present or not. Thus, the inhibitory effect of interferon on the generation of suppressor cells during the initial MLR did not appear to be because of cell destruction. The effect was reversible: when the cells were washed and restimulated, suppressor cell activity developed. We suggest, therefore, that in the primary MLR, interferon

inhibited the development of suppressor cell activity (perhaps by inhibiting the differentiation of presuppressor cells into suppressor cells). However, upon restimulation, the presuppressor lymphocytes escaped interferon's negative action and developed normal suppressor activity.

The inhibitory effect of interferon on the proliferation of allospecific suppressor T lymphocytes might explain some of its other actions during the allogeneic response, e.g., such as inhibition of MLR and enhancement of lymphocytotoxicity. We confirmed (2–5) in our system that interferon inhibited lymphocyte proliferation in a primary MLR.[2] This inhibitory effect might well be because of a direct action on the cell of all the lymphocytes subsets or possibly there might be a selective effect on suppressor cell division at low interferon doses (i.e., 10^1 to 10^2 interferon U/ml). In accord with this latter hypothesis, 10–30% of the proliferation observed during the MLR (Fig. 1) would represent proliferation of suppressor T lymphocytes.

In accord with previous work (3–6), we showed that interferon-treated lymphocytes harvested from the MLR showed an enhanced cytotoxicity. Our results do not permit us to distinguish between an increase in the number of cytotoxic lymphocytes or an increase in the cytotoxicity of individual lymphocytes. This increased cytotoxicity cannot be explained by increased natural killer cell activity because PHA-induced blasts resistant to natural killer cell lysis were used as target cells. It is possible that the increased cytotoxicity reflects either a direct effect on lymphocytes (6, 21) or that in the absence of suppressor cells more cytotoxic T lymphocytes differentiate, explaining, at least in part, these results.

The secondary proliferative responsiveness of lymphocytes primed in presence of interferon was also modified. Different results were observed when testing these primed cells in a secondary MLR (7, 8), either against the original stimulating cells (specific response) or against cells bearing irrelevant and serologically unrelated HLA-DR antigens (nonspecific response). We found that the seconday proliferation of memory lymphocytes specifically stimulated by the HLA-DR-priming antigens was only slightly affected by interferon (Fig. 2). On the other hand, the secondary proliferation of primed interferons that proliferated in the presence of cells carrying irrelevant antigens was decreased. Thus, lymphocytes primed in the presence of interferon generate memory cells that exhibit a very specific HLA-DR secondary proliferative response, i.e., a low response against irrelevant antigens.

It is known (16) that some primed lymphocytes are very specific, whereas others cannot be used in the PLT because of poor specificity of the proliferative response. The explanation for this phenomenon is not clear. Bach and co-workers (22) postulated that the nonspecific proliferative response of primed cells was because of the presence of shared determinants on the stimulating cells. Thus, the surface of stimulating cells might present a mosaic of epitopes shared by several specificities, and recent results with clones of primed lymphocytes favor this interpretation (23–25). However, the finding that interferon decreases nonspecific proliferative response is consistent with another hypothesis. The nonspecific recruitment of alloreactive "irrelevant" clones during the initial sensitization phase in the MLR might be because of mitogenic

[2] The inhibitory effect was related to the amount of interferon (Fig. 1), but it is interesting that individual donors appeared to differ in their sensitivity to this effect of interferon (data not shown). It has been reported that there is strain variability in mice in the sensitivity to the anti-proliferative action of interferon (17).

factors (17). According to this hypothesis, interferon might inhibit the production or action of such factors, thus limiting the expansion of the "irrelevant" clones responsible for the nonspecific response. It is not clear from the experiments reported here whether this postulated action of interferon is related to the inhibition of differentiation of suppressor lymphocytes or involves other mechanisms, such as a direct action on lymphokine-producing cells.

Interferon, therefore, profoundly modifies the alloimmune responsiveness of lymphocytes. Its effects would seem to render host defenses much more effective by increasing the duration and the specificity of lymphocyte response.

Summary

The effect of human interferon α on the differentiation of functional populations of lymphocytes during the human allogeneic response in vitro was studied. Interferon α inhibited the generation of allospecific suppressor T lymphocytes that normally develop from lymphocytes primed in vitro against allogeneic cells. This effect was not the result of the destruction by interferon of precursor suppressor cells but rather to inhibition of their differentiation into active suppressor T lymphocytes. This inhibition was reversible and could be overcome by repeated allogeneic stimulation even in the presence of interferon.

Inhibition of the generation of allospecific suppressor lymphocytes by interferon might play an important role in the allogeneic response. Interferon inhibited the proliferation of lymphocytes after allogeneic stimulation in a primary mixed lymphocyte reaction but enhanced their cytotoxicity. Despite the inhibitory effect in the primary mixed lymphocyte reaction, the specific secondary proliferative response of lymphocytes primed against a single HLA-DR antigen was only slightly affected by interferon. On the other hand, the nonspecific secondary proliferative response of lymphocytes primed in the presence of interferon was significantly reduced, indicating that interferon might decrease the recruitment of nonspecific "irrelevant" clones of responding cells during the sensitization period.

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