

CONTRASTING EFFECT OF α/β - AND γ -INTERFERONS ON
EXPRESSION OF MACROPHAGE Ia ANTIGENS

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Interferon (IFN)- γ is a T lymphocyte product that induces class II MHC products or Ia antigens on various cell types (reviewed in 1). This phenomenon was first described on macrophages (M ϕ), and it has not been mimicked by other types of IFN (2). However, macrophages can synthesize IFN- α/β after induction with such agents as LPS, poly-I:C, and virus infection (3, 4). We studied the effects of affinity-purified IFN- α/β on Ia induction by IFN- γ . We observed that murine IFN- α or - β markedly counteracted the induction of Ia antigens, as monitored by immunofluorescence and antigen presentation. Furthermore, the endogenous unresponsiveness of newborn mouse M ϕ cultures to IFN- γ was reversed in the presence of anti-IFN- β mAb.

Materials and Methods

Interferons. Murine IFN- α/β was purified from Newcastle Disease virus (NDV)-induced L-cell IFNs by using control pore glass (CPG) and polyclonal antibodies (mona 2 Δ ; generously donated by Drs. B. J. Dalton and K. Paucker, The Medical College of Pennsylvania, PA) to a sp act of 10^8 IU/mg. IFN- α and IFN- β were purified from NDV-induced mouse L-cell IFNs by CPG and 4E1 and 7FD9 mAb columns to specific activities of 10^8 and 5×10^8 IU/mg, respectively. Murine IFN- γ was prepared from the culture supernatant of an IL-2-dependent T cell line, B5, which has been stimulated with Con A as described previously (5). The supernatant was purified by CPG and phenyl-Sepharose to a sp act of 10^6 IU/mg. Subsequent to the completion of the experiments reported here, we have shown that rIFN- γ gave identical results to the B5-conditioned medium.

Anti-IFNs. 4E1 and 7FD9 are rat IgG₁ mAbs against mouse IFN- α and IFN- β (Watanabe, Y., in preparation). Both were purified by precipitation in 50% ammonium sulfate and DEAE-cellulose chromatography. Both mAbs would neutralize 10^4 IU of specific IFNs to 1 IU at 1 μ g/ml. The anti-IFN- γ reagent was serum from rats immunized with highly purified murine rIFN- γ (donated by Shionogi Laboratory, Osaka, Japan).

Induction of Ia Antigens. Peritoneal exudates were from 2-3-mo-old H-2^k (C3H/HeS1c, B10.BR, CBA/J) or H-2^d (BALB/c) mice that had been injected i.p. with 2 ml thioglycollate medium 4 d previously. Newborn exudates were from mice injected within 24 h after birth with 0.2 ml of thioglycollate (6). The exudates ($1-2.5 \times 10^6$ cells/dish) were adhered to 13-mm round glass coverslips that had been placed in 60-mm Petri dishes in 10% FCS in RPMI-1640. During the last 30 min of the 2-3 h adherence step, the cells were exposed to 20 μ g/ml mitomycin-C (Kyowahakko Co., Ltd., Tokyo, Japan) to prevent proliferation. The coverslips were washed to remove nonadherent cells, transferred into 24-well plates

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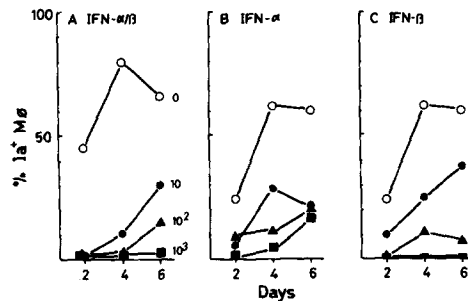


FIGURE 1. IFN- α and - β each blocks the Ia-inducing activity of IFN- γ . M ϕ were obtained from C3H/He (A) or B10.BR (B, C) mice that had been injected with thioglycollate medium 4 d previously. After washing off nonadherent cells and preventing cell growth with mitomycin C, we added IFN- γ (12.5 IU/ml) simultaneously with (A) IFN- α/β , (B) IFN- α , or (C) IFN- β at the indicated doses. The percentage of Ia⁺ M ϕ was determined at 2, 4, or 6 d.

(Nunc, Roskilde, Denmark), maintained up to 6 d in 1 ml medium with or without different IFNs and anti-IFNs, rinsed, and fixed with 1% paraformaldehyde. Ia antigens were visualized by indirect immunofluorescence (7). mAbs used for staining were 10.2.16 (Ia^k), MK-D6 (Ia^d), 2.4G2 (FcR), and F4/80 (M ϕ antigen), and were provided by Dr. R. M. Steinman (The Rockefeller University, New York). ≥ 200 cells were counted on duplicate coverslips and the results were recorded as the mean percentage of fluorescent cells.

Presentation of Ia Antigens. Presentation was assessed by the proliferation of (BALB/c \times DBA/2)F₁ (H-2^d) anti-B10.BR (H-2^k), allo-Ia-restricted, Lyt-2⁻ T blasts isolated from dendritic-Lyt-2⁻ T cell clusters (8). In these experiments, M ϕ were precultured for 3 d in 96 microtest plates (F96; Nunc) in medium with supplements described in Results. The cultures were rinsed, and half of the duplicates were fixed with 0.03% paraformaldehyde in PBS for 10 min at 20°C. 2×10^4 T blasts were added onto the monolayers. After 24 h in culture, 0.5 μ Ci [³H]TdR was added for 6 h.

Results

As has been described previously (2, 7) IFN- γ induced the expression of Ia antigens on 65–75% of cultured, thioglycollate-elicited M ϕ (Fig. 1). Peak expression was observed after 4 d of culture and an IFN- γ dose of 12.5 IU/ml. The Ia persisted on the cells for up to 6 d in the presence of IFN- γ (2, 7).

When highly purified IFN- α , - β , or their mixture was added to M ϕ cultures, Ia expression was blocked. Partial inhibition was observed by 3–100 IU/ml IFN- α and/or - β . Complete inhibition was seen at 100–1,000 IU/ml (Fig. 1 and Table I A). Large doses of IFN- γ , however, did not overcome the inhibitory effects of 1,000 IU/ml IFN- β (data not shown). IFN- β was fully inhibitory when given before or 12 h after the addition of IFN- γ , and was partially inhibitory when given later (Fig. 2 A). The inhibitory effects of IFN- β were reversible (Fig. 2 B).

Antibodies to IFN- β or - γ had the expected effects. Anti-IFN- γ blocked the inductive effect of IFN- γ , and anti-IFN- β blocked the inhibitory effect of IFN- β over a range of IFN titers, cell doses, and times in culture (Table I). Anti-IFN- β accelerated and prolonged the IFN- γ -induced Ia expression on adult M ϕ (Table I), suggesting that some endogenous IFN- β production may occur in adult M ϕ preparations. The addition of various IFNs or anti-IFNs singly or in concert did

TABLE I
Effects of IFNs and Anti-IFNs on Macrophage Ia Expression

Exp.	Number of M ϕ /cm ²	Day of assay	Percent Ia ⁺ M ϕ in cultures with:									
			IFN- γ	IFN- β	IFN- γ + IFN- β (IU/ml)					IFN- γ + anti-IFN- γ	IFN- γ + anti-IFN- β	IFN- γ + IFN- β + anti-IFN- β
					3	10	30	100	1,000			
A	5 \times 10 ⁴	2	28		27	20	13	4	0	0	51	
	5 \times 10 ⁴	4	67		51	48	42	14	0	0	63	
	5 \times 10 ⁴	6	48		41	41	30	0	0	0	60	
B	1.4 \times 10 ⁵	4	50	0					0		51	53
	4 \times 10 ⁴	4	53	0				2			50	53
	1.4 \times 10 ⁴	4	52	0				1			54	50

M ϕ were obtained from C3H/He mice (by adherence of thioglycollate-elicited peritoneal exudates onto glass coverslips) and cultured with or without IFN- γ (12.5 IU/ml), IFN- β (1,000 IU/ml), and anti-IFN antibodies. Anti-IFN- β and anti-IFN- γ were mixed with the IFNs in amounts capable of neutralizing 1,000 IFN- β and 15 IU IFN- γ , respectively, and incubated for 30 min at 4°C before addition in the culture. Data are percent of Ia⁺ M ϕ .

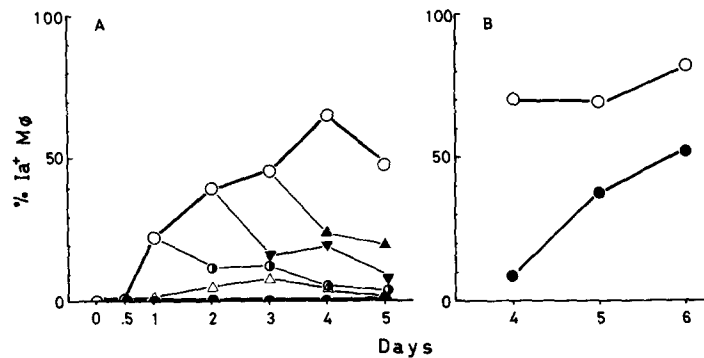


FIGURE 2. (A) Ia induction when IFN- β is added after IFN- γ . After stimulation of C3H/He M ϕ with IFN- γ at 12.5 IU/ml (O), IFN- β at 1,000 IU/ml was added at 0 (●), 12 (Δ), 24 (○), 48 (∇), or 72 (\blacktriangle) h. (B) Reversibility of the IFN- β block. M ϕ from CBA/J mice were cultured with (●) or without (○) 1,000 IU/ml IFN- β for 24 h, washed, and then cultured with IFN- γ for 4, 5, or 6 d in the absence or IFN- β . Ia⁺ M ϕ were determined at each time point.

not alter M ϕ viability, as indicated by trypan blue exclusion and expression of M ϕ surface antigens (See Materials and Methods.)

The capacity of Ia⁺ M ϕ to stimulate helper T cells was evaluated by using Lyt-2⁻ alloreactive T blasts. Ia⁺ M ϕ , even when fixed with paraformaldehyde, stimulate T blasts but not resting T cells (8, 9). The stimulatory capacity of M ϕ that had been cultured with IFN- α or - β , or their respective Abs, varied according to the number of Ia⁺ cells in the monolayers (Fig. 3). Viable Ia⁻ M ϕ populations were only stimulatory at high cell doses, suggesting that some Ia may have been induced during the culture period with T cells.

Given the effects of exogenous IFNs, we wondered whether there were situations where endogenous IFNs could block the effect of IFN- γ . We have been unable to induce Ia in newborns' M ϕ with IFN- γ (Table II A), even when the cultures were supplemented with 1 μ g/ml indomethacin (data not shown). Furthermore, admixing newborn and adult M ϕ blocked the response of adult cells to IFN- γ (Table II B). In the presence of anti-IFN- β mAb, newborn M ϕ became responsive to IFN- γ , and their inhibitory activity for adult M ϕ was also counteracted (Table II). Anti-IFN- α in contrast had no effect (not shown). The

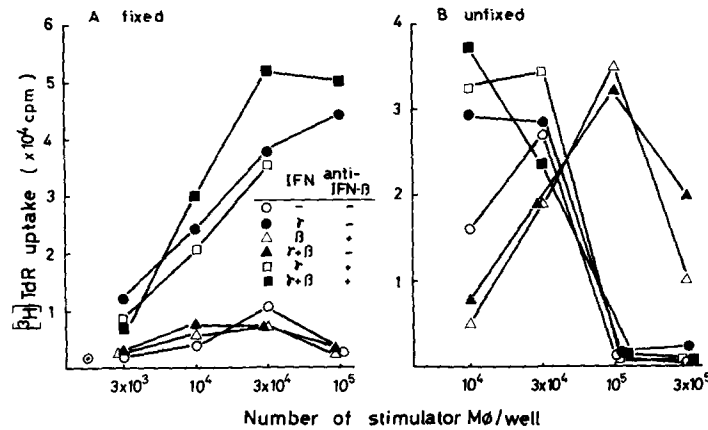


FIGURE 3. Effect of IFNs and anti-IFN- β on the presentation of M ϕ Ia. CBA/J M ϕ were cultured for 3 d in 96 microtest plates with different combinations of IFNs and anti-IFNs, as indicated in the key. After washing, one-half of the M ϕ cultures were fixed with 0.03% paraformaldehyde (A), while another half was not (B). 2×10^4 Lyt-2 $^-$, Ia k -restricted, T blasts were then added to these M ϕ , and [3 H]TdR uptake was measured at 24–30 h. Symbols show geometric means of triplicate cultures. All numbers on the abscissa represent the number of peritoneal exudate cells initially plated, of which ~40% remained adherent at the time the T blasts were added. 50–70% of the M ϕ cultured with IFN- γ were Ia $^+$.

TABLE II
Unresponsiveness of Newborn M ϕ Is Due to Presence of Endogenous IFN- β

Exp.	Reagent	Percent Ia $^+$ M ϕ					
		Newborn (H-2 k) on day:			Adult (H-2 k) on day:		
		2	4	6	2	4	6
A	—	0	0	0	0	0	0
	IFN- γ	0	1.2	1.0	41.3	66.4	45.7
	Anti-IFN- β	0	0	0	0	0	0
	IFN- γ + anti-IFN- β	14.1	33.9	41.3	60.2	78.4	55.0
B		Adult (H-2 k)		Adult		Adult	Newborn
				H-2 k	H-2 d	(H-2 k)	(H-2 d)
	—	2.9		0.8	0	0.7	0
	IFN- γ	69.1		33.5	33.1	6.8	3.9
	Anti-IFN- β	0.6		0.7	0	0.3	0
IFN- γ + anti-IFN- β	69.2		33.4	33.9	33.9	19.4	

M ϕ were obtained from newborn and adult C3H/He mice (A), or newborn BALB/c mice and adult C3H/He or BALB/c mice (B). Each M ϕ preparation, or the mixtures (1:1), was cultured with reagents as indicated in Table I. The percentage of Ia $^+$ M ϕ was determined at 2, 4, and 6 d in (A) or at 4 d in (B) using 10-2.16 (anti-Ia k) or B21-2 (anti-Ia d) mAb.

distinction between newborn and adult M ϕ in cocultures of the two populations was accomplished by using cells from H-2 k and H-2 d mice for which we had specific anti-Ia mAb (Table II B).

We further evaluated the effects of newborn M ϕ -conditioned medium on IFN- γ induction. The supernatants were markedly inhibitory, and the inhibition was reversed with anti-IFN- β . The titer of IFN- β in the supernatant was only <30–50 U/ml. This level is lower than the level of affinity-purified IFN that is required to block IFN- γ (Fig. 1 and Table I). This raises the possibility that some factor(s) other than IFN- β , e.g., colony-stimulating factor (CSF-1) made by

contaminating fibroblasts, may play an intermediary role in inducing adult M ϕ to synthesize IFN- β (10). Our newborn M ϕ preparations contained >80% phagocytes by latex ingestion, and 15–20% fibroblasts by morphology.

Discussion

Although IFN- α , - β , and - γ all induce resistance to viruses and augment the level of several membrane-associated antigens such as FcR and MHC class I antigens on M ϕ (reviewed in 1), their effects on the expression of Ia are quite different. IFN- γ induces Ia, while IFN- α and - β do not induce Ia but instead block the inductive effect of IFN- γ . During the preparation of this manuscript, Ling et al. (11) independently made some similar findings. Adult M ϕ were pretreated with IFN- β for 24 h, and then stimulated with IFN- γ for 48 h. A partial inhibition of the IFN- γ induction of Ia was observed. Our data confirm those of Ling et al. In addition, we have shown that the effect of IFN- α/β is marked (Fig. 1) and longlasting (Fig. 2B), is evident at the level of Ia expression and antigen presentation (Fig. 3), and is observed even when IFN- β is added 2–3 d after the IFN- γ (Fig. 2A).

Immunosuppression has been observed after the administration of agents that induce IFN- α/β , such as certain adjuvants. IFN- α/β are known to be suppressive for immune responses, including antibody responses, delayed hypersensitivity, and graft rejection (1, 12). Newborn M ϕ lack Ia (13) and can be immunosuppressive (14). From our findings, such immunosuppressive effects may be influenced by the negative regulation of Ia expression by IFN- β , thereby reducing the ability of M ϕ to interact with primed T cells and enhance immune responses (7–9). Our observations pose an interesting paradox for the antigen-presenting capacity of M ϕ , since these cells are excellent producers of IFN- α/β (3, 4) and may therefore self regulate their levels of Ia.

Our current studies explore the mechanisms of IFN action at the level of IFN- γ -R and Ia synthesis. The effects of lymphokines on M ϕ Ia are likely due to an induction in the synthesis of Ia, and are known to be reversible (15). We are finding that the fall in Ia that occurs upon removal of IFN- γ is more rapid when IFN- β is present. This suggests that IFN- β could have direct effects on the synthesis and/or expression of macrophage Ia.

Summary

IFN- γ is known to induce the expression of Ia antigens on macrophages. We found that murine IFN- α and - β blocked the effects of IFN- γ in a dose-dependent manner. The antagonistic effect of IFN- α and - β was observed even when macrophages were prestimulated with IFN- γ . These inhibitory effects of IFN- α or - β were blocked by their respective antibodies. The block exerted by IFN- α/β was similar whether Ia levels were monitored by immunofluorescence with anti-Ia mAb, or by stimulation of freshly sensitized, alloreactive T lymphoblasts.

Adherent macrophage-rich populations from newborn mice were incapable of expressing Ia antigens following stimulation with IFN- γ , and would inhibit the response of adult macrophages to this lymphokine. Addition of anti-IFN- β mAb, but not anti-IFN- α allowed newborns' macrophages to express Ia in response to IFN- γ , and ablated the suppressive activity toward adult cells.

These results indicate that IFN- α and - β , which can be produced in the course of self-defense responses and during ontogeny, may contribute to the down-regulation of macrophage Ia expression.

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