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Recombinant Mouse Interferon-γ Regulation of Antibody Production

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Interferon-γ produced in monkey cells by transfection with mouse interferon-γ cDNA suppressed the mouse in vitro antibody response in a manner similar to that of natural mouse interferon-γ. Significant suppression was obtained with as little as 1 U of interferon. Recombinant human interferon-γ produced by cloning in a similar fashion was not suppressive. Both the suppressive and the antiviral activities of recombinant interferon-γ were neutralized by antibodies to mouse natural interferon-γ. Thus, interferon-γ was responsible for the immunosuppression. At the cellular level, the recombinant interferon-γ was capable of activating macrophages to suppress antibody production. The data provide clear-cut evidence that interferon-γ plays an important role in regulation of immunological processes.

Interferons (IFNs) are a family of functionally similar molecules that appear to play an important role in the regulation of cellular activities. Important biological activities that are modulated by IFN are virus replication and the immune response (2, 9). IFNγ, a product of T cells, has been more difficult to produce in large quantity and purify to homogeneity than IFNα and β. Additionally, the cDNAs of IFNα and β have been expressed in bacteria (3, 6, 18, 23), and the products have been used to verify some of the biological functions of natural IFNs. Recently, both human and mouse IFNγ cDNAs have been expressed in *Escherichia coli* and monkey cells (7; P. W. Gray, S. H. Lee, and D. V. Golddel, The Third Annual International Congress for Interferon Research, Miami, 1982). We report here that mouse IFNγ produced by expression of the IFNγ cDNA in monkey cells is a potent regulator of the in vitro antibody response, thus providing clear-cut evidence that IFNγ regulates B-cell function, as suggested by previous studies with crude IFNγ preparations (11, 15, 17, 21).

MATERIALS AND METHODS

Mice. C57B1/6 female mice, 8 to 12 weeks old, were obtained from Jackson Laboratories, Bar Harbor, Maine.

IFNs. Recombinant mouse (MoIFNγ) and human (HuIFNγ) IFNγ that were similarly produced by transfection of monkey cells with the appropriate cDNAs (7; Gray et al. The Third Annual International Congress for Interferon Research) were generously supplied by Genentech, Inc. (South San Francisco, Calif.). Each preparation contained approximately 1,000 U of IFN activity per ml. Natural MoIFNγ was produced in mouse spleen cell cultures as described previously (4) and partially purified to approximately 10^6 U/mg of protein by sequential chromatography on controlled pore glass beads, Ultrogel AcA 54 (LKB), and concanavalin A–Sepharose (4).

IFN assay and neutralization by specific antibody. MoIFNγ was assayed by a microplaque reduction method with approximately 40 PFU of vesicular stomatitis virus per well in mouse L cells (16). HuIFNγ was assayed on WISH cells by a cytopathic assay with Sindbis virus (1). In our studies, a concentration of 1 U of IFNγ per ml is defined as the concentration required to decrease the number of PFU per well or the cytopathic effect by 50%. One unit of our IFNγs approximated the antiviral activity of 1 U of NIH reference fibroblast cell IFN (IFNα and β) standards for both mouse and human IFNs. Antibody neutralizations were carried out as described previously (19) with excess antibody that was produced to natural MoIFNγ, and normal rabbit serum was the negative control.

Culture. Dissociated mouse spleen cells were cultured in duplicate or triplicate for in vitro direct plaque-forming cell (PFC) responses to sheep erythrocytes (SRBC) for 5 days at 1.5×10^7 cells in 1 ml (14). Recombinant or partially purified IFNγ and IFNγ-treated macrophages were added to cultures at day 0. Direct PFC assays were carried out on microscope slides as described previously (14).

RESULTS AND DISCUSSION

We first compared recombinant MoIFNγ with natural MoIFNγ for their relative abilities to suppress the in vitro antibody response of spleen cells from C57B1/6 mice. Both sources of IFNγ were potent suppressors of the in vitro PFC response to SRBC (Table 1, experiment 1). Thus, recombinant MoIFNγ was as potent a suppressor of the antibody response as was

TABLE 1. Suppression of the mouse in vitro anti-SRBC PFC response by mouse recombinant and natural IFNγ

Expt	Treatment[a]	IFN (U/ml)	Direct PFC/culture ± SD	% Suppression
1	Recombinant MoIFNγ	33	110 ± 70	97
		1	907 ± 201	73
	Natural MoIFNγ	61	165 ± 106	95
		6	1,440 ± 640	56
	None (control)		3,307 ± 378	
2	Recombinant MoIFNγ	33	1,483 ± 837	75
	Recombinant HuIFNγ	30	10,720 ± 3,431	-83
	None (control)		5,867 ± 281	
3	Recombinant MoIFNγ	100	3,147 ± 805	79
	Recombinant MoIFNγ plus Anti-IFNγ	<3	14,667 ± 2,001	1
	Recombinant MoIFNγ plus normal rabbit serum	100	2,773 ± 1,201	81
	Anti-IFNγ		11,600 ± 1,697	18
	Normal rabbit serum		14,027 ± 1,361	6
	None (control)		14,880 ± 2,080	
4	Macrophages treated with recombinant MoIFNγ	<3	1,120 ± 423	71
	Untreated macrophages	<3	3,840 ± 1,466	
	Control		3,093 ± 489	

[a] Treatments were as described in the text. Peritoneal macrophages from unstimulated C57B1/6 mice were incubated at 1×10^6/ml with a final concentration of 100 U of recombinant MoIFNγ per ml for 4 h. The residual MoIFNγ was removed by washing the cells twice before adding them at a final concentration of 5×10^5/ml to spleen cell cultures. Suppression of the PFC response by MoIFNγ-treated macrophages is based on the untreated macrophage control.

natural MoIFNγ. We tested the effect of HuIFNγ (also produced by transfection of monkey cells) on the mouse anti-SRBC PFC response to eliminate the possibility that factors from monkey cells other than MoIFNγ were responsible for the immunosuppression. Recombinant HuIFNγ did not suppress the anti-SRBC PFC response (Table 1, experiment 2). Recombinant MoIFNγ at a similar concentration was a potent inhibitor of the PFC response. Although the antibody response in the presence of recombinant HuIFNγ was higher than that of the control, the PFC responses in the presence of recombinant HuIFNγ were similar to control responses in repeated experiments. Since the MoIFNγ and HuIFNγ cDNAs were incorporated into similar vectors (7), MoIFNγ was probably responsible for the observed immunosuppression. The immunosuppressive effects of MoIFNγ on antibody production was observed in over six different experiments.

To further determine the relationship of the recombinant and natural MoIFNγ immunosuppressions, antiserum to natural MoIFNγ (19) was tested for its ability to neutralize both the antiviral and immunosuppressive properties of recombinant MoIFNγ. Anti-MoIFNγ in excess completely neutralized both the antiviral and the PFC suppressive effects of recombinant MoIFNγ (Table 1, experiment 3), thus providing additional evidence that MoIFNγ is a potent immunoregulatory lymphokine. The anti-MoIFNγ had no effect on the antiviral or immunosuppressive properties of MoIFNα and β (data not shown).

A dose-response curve for recombinant MoIFNγ is presented in Fig. 1. In general, we obtained 50 to 80% suppression of the mouse anti-SRBC PFC response with as little as 1 U of

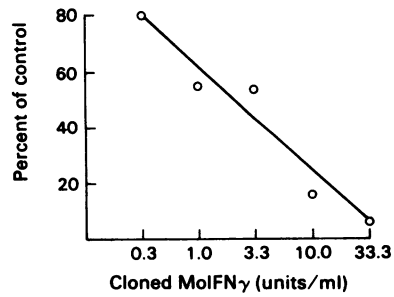

FIG. 1. Dose-response curve for recombinant MoIFNγ suppression of the anti-SRBC PFC response of C57BL/6 spleen cell cultures. MoIFNγ was added to cultures at day 0 of 5-day cultures.

recombinant or partially purified natural MoIFNγ per ml. Thus, recombinant and partially purified natural MoIFNγ appear to be more potent immunoregulators than MoIFNα or β, since 20 to 100 U of MoIFNα or β per ml is usually required for similar suppression (14), which was again confirmed (data not shown). This greater potency of MoIFNγ for immunosuppression has previously been suggested (21).

The cellular sites of action of IFN in regulating antibody production are multiple, involving B cells, T cells, and perhaps macrophages (5, 8, 10, 13). Suppression can also occur through induction of suppressor cell and suppressor factor activity (12). We have preliminary data that suggests that recombinant MoIFNγ can suppress antibody production by acting on B cells, T cells, or macrophages. MoIFNγ activity on macrophages may occur through inducing them to become suppressor cells. Treatment of macrophages with recombinant MoIFNγ, followed by removal by washing and addition of these treated macrophages to normal spleen cell cultures, resulted in significant suppression of the anti-SRBC PFC response (Table 1, experiment 4). MoIFNγ can thus induce or activate macrophages to suppress antibody production.

Both partially purified and recombinant MoIFNγ have recently been shown to induce expression of Ia antigens on macrophages (22) and to activate macrophages for enhanced killing of tumor cells (19a). How these functions of MoIFNγ are related to regulation of antibody production remains to be determined. Macrophages are known to both help and suppress antibody production (20). It would be of interest to determine whether the mechanism by which IFNγ modulates macrophage regulation of antibody formation is related to Ia antigen expression or activation of macrophages. Certainly, IFNγ appears to play a central role in the regulation of immunological processes related to antibody production.

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