Interferon Immunosuppression: Mediation by a Suppressor Factor

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Suppression of the in vitro antibody response to sheep erythrocytes by mouse fibroblast interferon occurred by induction of suppressor cell activity in spleen cells. The suppressor cells produced a soluble factor which mediated the immunosuppression. The suppressor factor did not inhibit virus replication; thus, interferon probably regulates the B-cell response by a mechanism that is different from its antiviral effect.

Interferon can play an important role in regulation of the antibody response in doses that occur naturally (3, 5, 9). We have recently shown that interferon can exert its antiviral effect indirectly through a cell-to-cell transfer mechanism (1, 2), but it is not clear as to whether interferon has a direct or indirect effect on the B-cell response (5-7). Further, it is not clear that the mechanism by which interferon suppresses the immune response is the same as (or different from) that by which it blocks virus replication. There are preliminary data that suggest that virus-induced (fibroblast) interferon exerts its immunosuppressive and antiviral effects by different mechanisms in the mouse system (6, 8). In this report, we propose to determine whether interferon can block B-cell function indirectly and, if so, to determine the mechanism of this blockage.

MATERIALS AND METHODS

Mice. C57BL/6 female mice, 8 to 12 weeks old, were obtained from Jackson Laboratories, Bar Harbor, Maine.

Antigens. Sheep erythrocytes were obtained from Colorado Serum Co., Denver, Colo.

Diluent. Antigens, spleen cells at the time of harvesting, and cell supernatants were all suspended or diluted in modified minimal essential medium (11).

Cultures. Dissociated mouse spleen cells were cultured for in vitro plaque-forming-cell (PFC) responses to sheep erythrocytes as previously described (11). Cultures consisted of 1.5×10^7 spleen cells per ml. All PFC responses were determined on day 5. Direct PFC assays were carried out on microscope slides. All results are expressed as the average of duplicate or triplicate cultures.

Interferon assays. A microplaque reduction assay for interferon was performed as described previously using vesicular stomatitis virus (40 plaque-forming units per well) (10).

Interferon. Mouse fibroblast interferon from L cells (400,000 NIH reference units per ml) and mouse fibroblast interferon from C-243 cells (30,000 NIH

reference units per ml) were prepared as previously described (9).

Antibody to interferon. Rabbit anti-mouse L-cell interferon was supplied by the Antiviral Substances Program, National Institute of Allergy and Infectious Diseases, Bethesda, Md. (12). The titer was 1:6,000, the highest dilution in 1 ml that neutralized 10 U of L-cell interferon.

Interferon induction of suppressor cells. Spleen cells (2.5×10^7 to 1×10^8 /ml) were incubated with various concentrations of interferon for 2 or 24 h, after which the cells were washed twice with modified minimal essential medium and suspended to the desired concentration in culture medium. Suppressor cells were either added directly to the in vitro PFC culture system or treated as described in Results and Discussion and then added to the system.

Suppressor factor. Suppressor cells obtained by 24-h treatment with interferon and washed and suspended in culture medium were the source of suppressor factor. Cells (10^6 /ml) were incubated at 37°C for 2 h, after which they were centrifuged at 1,500 rpm in an RC-3 centrifuge (Sorvall) (HR-8 head). The supernatant obtained is referred to as suppressor factor. Suppressor factor was either added directly to the in vitro PFC system or treated as described in Results and Discussion and then added to the system.

RESULTS AND DISCUSSION

We first addressed the question of whether interferon can block B-cell function indirectly by possible induction of suppressor cell activity in C57BL/6 female mouse spleen cell cultures. This was tested by treating spleen cells with interferon and adding the treated cells to untreated spleen cells. Spleen cells were treated with L-cell fibroblast interferon for 24 h. The cells were washed twice to remove the interferon and then added to syngeneic cultures of untreated spleen cells along with sheep erythrocyte antigen. Suppressor cell controls consisted of spleen cells incubated with culture medium alone. Significant suppression of the in vitro direct PFC response after 5 days of incubation

was observed with cells treated with two concentrations (500 and 5,000 U/ml) of interferon and with two concentrations of suppressor cells (1×10^7 and 3×10^6 /ml) (Table 1). The PFC responses of the cultures to which the suppressor cells were added were inhibited by approximately 90%, whether expressed as PFC per culture or PFC per 10^6 viable cells. Over 90% of the interferon used to treat the spleen cells was recovered. Under conditions where 1×10^8 cells were treated with 500 U of interferon, the addition of 3×10^6 of these washed cells to the PFC cultures would have resulted in a maximum carry-over of only 2 U of cell-bound interferon. We previously determined that at least 50 U of interferon was required in the cultures to suppress the PFC response by 90%. Thus, interferon appears to induce suppressor cell activity. Suppressor cell activity can be induced with as little as 100 U of interferon per ml and with treatment as short as 2 h. Conditions for interferon induction of suppressor cell activity, then, correspond to those for interferon suppression of the immune response (9). Cell viabilities for both interferon-treated cells and untreated controls were essentially the same, i.e., 75 to 85%. Interferons of specific activities of 10^5 and $10^{8.5}$ U/mg of protein were both capable of inducing suppressor cell activity.

Further characterization of the interferon-induced suppressor cell activity was done by treatment of interferon-induced suppressor cells with antibody to mouse L-cell fibroblast interferon to remove residual interferon. Results are shown in

TABLE 1. *Effect of interferon-treated mouse spleen cells on in vitro anti-sheep erythrocyte PFC response of untreated cells^a*

Interferon (U/ml)	No. of treated spleen cells added	PFC per culture $\pm$ SD ^b	PFC per 10^6 viable cells $\pm$ SD
5,000	1×10^7	330 $\pm$ 297	156 $\pm$ 134
	3×10^6	1,260 $\pm$ 594	853 $\pm$ 810
500	1×10^7	440 $\pm$ 452	190 $\pm$ 228
	3×10^6	2,580 $\pm$ 481	1,284 $\pm$ 478
0	1×10^7	3,520 $\pm$ 453	2,005 $\pm$ 1,061
	3×10^6	8,200 $\pm$ 2,942	3,799 $\pm$ 1,797

^a C57BL/6 spleen cells (10^8 /ml) were incubated with the indicated concentrations of interferon for 24 h. After washing, 1×10^7 (100 μ l) or 3×10^6 (33 μ l) cells were added to fresh spleen cell cultures containing 1.5×10^7 syngeneic cells in 1 ml. Sheep erythrocytes were added, and the cultures were incubated for 5 days, after which the direct anti-sheep erythrocyte PFC response was determined.

^b SD, Standard deviation.

Table 2. Specific antibody did not block or reduce the suppression, which was essentially 100%, and was comparable to normal rabbit serum and medium controls. As expected, spleen cells not treated with interferon were not suppressive under the same conditions. Further, the

TABLE 2. *Effect of antibody to interferon on suppressor cell and suppressor factor activities^a*

Condition	Treatment	Anti-sheep erythrocyte PFC per culture $\pm$ SD ^b	Inhibition (%)
Suppressor cells (5×10^6 /ml)	Anti-interferon	30 $\pm$ 14	99.7
	NRS	10 $\pm$ 14	99.7
	None	10 $\pm$ 14	99.9
Control cells (5×10^6 /ml)	Anti-interferon	7,980 $\pm$ 1,216	25
	NRS	4,600 $\pm$ 622	-3
	None	6,120 $\pm$ 113	17
Suppressor factor (1:10 dilution)	Anti-interferon	170 $\pm$ 113	98
	NRS	150 $\pm$ 212	97
	None	60 $\pm$ 57	99
Control factor (1:10 dilution)	Anti-interferon	10,200 $\pm$ 792	4
	NRS	6,320 $\pm$ 4,130	-41
	None	5,140 $\pm$ 990	30
Interferon (100 U/ml)	Anti-interferon	6,340 $\pm$ 1,500	41
	NRS	1,040 $\pm$ 962	77
	None	1,840 $\pm$ 509	75
None	Anti-interferon	10,680 $\pm$ 3,564	
	NRS	4,480 $\pm$ 792	
	None	7,380 $\pm$ 537	

^a Suppressor cells (interferon-treated) and control cells (untreated) were produced under conditions described in Table 1, footnote *a* using 1,000 U of interferon per ml with 24 h of incubation. Supernatants were obtained by incubating the washed cells (10^8 /ml) at 37°C for 2 h. Before addition to syngeneic cultures, suppressor cells (5×10^7 /ml), control cells, supernatants, and the interferon (1,000 U/ml) used for induction were incubated with equal volumes of a 1:20 dilution of anti-interferon serum, normal rabbit serum (NRS), or culture medium for 1 h at room temperature. The values shown represent the final cell, supernatant, and interferon concentrations added to syngeneic cultures.

^b SD, Standard deviation.

immunosuppressive effects of the interferon used to induce the suppressor cells were significantly blocked by preincubation of this antibody with interferon, which is consistent with previous observations. Thus, the suppressor cell activity was induced by interferon, did not require the continued presence of interferon, and could be attributed to either a direct or indirect action of suppressor cells.

Dose-response studies indicated that 1×10^5 to 3×10^5 suppressor cells per 1.5×10^7 untreated spleen cells resulted in significant suppression of the PFC response (data not shown). The high ratio (approximately 100:1) of untreated cells to suppressor cells that resulted in suppression of the PFC response suggested that direct cell-to-cell contact of the effector (suppressor) and responder (untreated) cells was not required. Further, this suggested that a mediator derived from the suppressor cell population was probably responsible for the suppression. Direct evidence for such a mediator was obtained by incubating high concentrations of interferon-treated cells for 2 h at 37°C and adding the supernatants to untreated cultures (Table 2). The PFC response was suppressed over 95%, and the suppression was not affected by prior incubation of suppressor supernatants with antibody to interferon, thus providing further evidence that interferon was not directly involved in the suppression. The suppressor factor may be a macromolecule since it did not pass through an Amicon filter with a molecular weight cutoff of 10,000, and, in fact, was concentrated under these conditions. Thus, interferon induced suppressor cells, which in turn produced a suppressor factor that was capable of suppressing the *in vitro* PFC response.

It was of interest to ascertain whether the suppressor factor possessed antiviral activity. Undiluted suppressor factor preparation contained antiviral activity equivalent to 10 to 30 U of interferon per ml, which could be residual after washing. All of this antiviral activity was neutralized by antibody to interferon (Table 3), which failed to neutralize the suppressor activity (Table 2; see immunosuppression by suppressor factor after treatment with anti-interferon). Interferon-induced suppressor factor, then, was active in suppression of the PFC response, but lacked antiviral properties.

Titration of the factor showed a linear relationship between inhibition of PFC response and suppressor factor dilution (Fig. 1). Induction of suppressor factor by interferon was completely blocked if the interferon was first neutralized by specific antibody before addition to spleen cell cultures. This is evidence that interferon was

TABLE 3. Antiviral activity of interferon-induced suppressor factor

Supernatant	Treatment	Interferon (U/ml)
Suppressor factor ^a	None	30 ^b
	Anti-interferon ^c	<3 ^b
Control suppressor factor ^d	None	<3 ^b
Interferon recovered after suppressor cell induction ^e	None	1,000
Interferon concn used to induce suppressor cells	None	1,000

^a Suppressor factor obtained from cells treated with 1,000 U of interferon per ml, washed, suspended to 10^6 cells per ml, and incubated at 37°C for 2 h.

^b Antiviral activity equivalent in units of interferon per milliliter.

^c Equal volumes of suppressor factor (undiluted) and antibody to interferon (1:20 dilution) were incubated together for 1 h at room temperature before titration for antiviral activity.

^d Control suppressor factor obtained from cells incubated in culture medium, washed, suspended to 10^6 cells per ml, and incubated at 37°C for 2 h.

^e Residual interferon concentration after treatment of cells for suppressor cell induction.

FIG. 1. Titration of interferon-induced suppressor factor. Suppressor factor was induced by treatment of 5×10^7 cells per ml with 1,000 U of interferon per ml for 24 h at 37°C. The washed cells (1×10^6 /ml) were incubated in culture media for 2 h at 37°C. The resultant supernatant was titrated against syngeneic cells and sheep erythrocytes for its ability to inhibit the PFC response. Symbols: ○, supernatant from cells treated with 1,000 U of interferon per ml; ●, supernatant from cells treated with 1,000 U of interferon per ml that had been neutralized by anti-interferon serum before addition to the cultures.

the inducer of the suppressor factor. In addition, it provides further evidence that the suppressor factor is not interferon.

The induction of a suppressor factor by interferon, which lacks antiviral activity, is consistent with two previous observations which suggest dissociation of the antiviral and immunoregulatory actions of interferon. One is the observation that the immunosuppressive effects of fibroblast interferon are blocked by 2-mercaptoethanol, whereas the antiviral property is unaffected (6). The other is that a ribosome-associated factor(s) obtained from interferon-treated cells is immunosuppressive, but lacks antiviral properties (8). One of the biochemical effects of interferon on cells has recently been shown to be a block of protein synthesis via blockage of formation of the initiation complex through ribosome-associated protein kinase activity (4, 13, 14, 16). To date, the only biological function that this mechanism has been shown to possibly affect is suppression of the immune response (8). It is quite possible, then, that interferon-induced molecular events, such as inhibition of initiation complex formation and suppressor factor induction, may be related to the nonantiviral properties of interferon.

Depletion of macrophages from the suppressor cell preparation by glass bead-glass wool columns (7) did not affect the suppressor cell activity (Table 4), which suggests that the suppressor cell is a lymphoid cell. Preliminary treatment of suppressor cells with anti-Thy-1 serum to remove T-cell activity and with rabbit anti-mouse immunoglobulin serum to remove B cells with surface immunoglobulins did not affect the suppressor cell activity (data not shown). Thus, the suppressor cell may be a null cell. This is consistent with previous studies (5, 7) involving B cell preparations containing null cells, which suggested that the B cell was the target for interferon suppression of the antibody response.

We have the following working model of the production and function of the suppressor factor. It is induced in spleen cell cultures by interferon. Its induction is blocked by treatment of interferon with specific antibody, but the immunosuppression by induced suppressor factor is unaffected by antibody to interferon. The factor is devoid of antiviral activity, which suggests that interferon regulates the immune response by a mechanism(s) that is different from its antiviral property. This differentiates the cell interactions that are involved in immunosuppression by interferon from the cell-to-cell interactions that are associated with the transfer of viral resistance (1). Additionally, efficient transfer of viral resistance requires cell-to-cell contact, which is

TABLE 4. *Effect of macrophage depletion on immunosuppressive effect of interferon-treated spleen cells^a*

Macro- phage depletion	Interferon treatment	Viable cells per ml added to cul- tures	Anti-sheep erythrocyte PFC per cul- ture $\pm$ SD ^b	Inhi- bi- tion (%)
Not depleted	Treated	8×10^6	$1,200 \pm 368$	88
	Untreated	8×10^6	$9,860 \pm 764$	
	Treated	4×10^6	$2,400 \pm 368$	62
	Untreated	4×10^6	$6,320 \pm 735$	
Depleted	Treated	8×10^6	530 ± 184	93
	Untreated	8×10^6	$7,560 \pm 113$	
	Treated	4×10^6	$1,640 \pm 820$	71
	Untreated	4×10^6	$5,640 \pm 113$	

^a Suppressor cells were induced with interferon as described in Table 1, footnote *a* (1,000 U, 24 h). Macrophages were depleted by passing the cells through a glass wool-glass bead column. The macrophage-depleted cells were washed twice before addition to syngeneic cultures at the indicated concentrations.

^b SD, Standard deviation.

not required in immunosuppression. Future studies will determine more definitively the nature of the suppressor cell and the relationship of interferon-induced suppressor factor to other mediators of suppression (15). Other types of interferons will also be tested for their ability to induce this factor. This suppressor factor may play a natural role both in normal immune mechanisms and in the host response to viral infections. It may be a desirable means of suppressing the immune response under certain conditions.

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