

Modulation of Immune Responses in Newborn and Adult Mice by Interferon

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Interferon was found to have both suppressive and enhancing effects on the antibody response in newborn and adult mice. Evidence was obtained that these effects are primarily evoked during the initial steps controlling cell proliferation. Stimulation of thymus and spleen cells with a T-cell mitogen was enhanced by low doses and suppressed by high doses of interferon. Treatment of parental spleen cells with interferon before injecting them into immunized F1 hybrid mice resulted in an enhanced allogeneic effect. These results are compatible with the hypothesis that interferon affects T cells and has an immunoregulatory role, either by inhibiting the action of suppressor cells or by promoting immunological maturation.

During recent years a multitude of effects have been ascribed to interferon (IF). In addition to the well-known antiviral effect, inhibitory and enhancing effects on different normal and neoplastic cells have been reported (8). As regards immune responsiveness, most reports have described suppressive effects of IF, but enhancing effects may be demonstrable in certain situations. In fact, IF has been suggested to act as an immunoregulatory substance (3, 14). Recent evidence suggests that the effector molecule of suppressor T cells is an IF, which may thus have a regulatory role on the immune response (4, 13).

As for the immunostimulatory effect of IF, no explanation is readily available for its mechanism of action. Possible causes of the enhancing effect might be stimulation of macrophage function (12) or enhanced expression of antigenic determinants on cell membranes (16). In the present report, we describe experiments designed to further elucidate the stimulatory effect of IF on antibody production in mice. Evidence is presented that the enhancing effect of IF on antibody-producing cells is indirect and apparently mediated by T cells. The effect may be exerted either as a consequence of suppressor cell inactivation or as a result of promoted immunological maturation of T cells.

MATERIALS AND METHODS

Mice. Albino mice obtained from Carworth Farms, N.J. (CFW), or inbred mice of the CBA, DBA/1J, C57Bl/6J strains or the F1 hybrids DBA/1J × C57Bl/6J were used in the experiments.

IF preparations. An IF preparation produced by C-243 cells and purified, as described by Tovey et al.

(24), to a specific activity of 5×10^6 U/mg of protein, was kindly supplied by M. Tovey. In some control experiments, mouse IF and mock IF obtained from Bionetics (Kensington, Md.) were used. Although it is recognized that the IF preparations used contained substantial amounts of contaminating proteins, they are herein referred to as IF only. IF assays were performed on mouse L cells inoculated with vesicular stomatitis virus, using microtiter plates, essentially as described by Havell and Vilček (9). The route of injection of IF and cells was intraperitoneal in newborn and 2-week-old mice and intravenous in adult mice.

Determination of antibody PFC. The hemolytic plaque assay described by Cunningham and Szenberg (5) was used. Indirect (immunoglobulin G) plaque-forming cells (PFC) were assayed after incorporation of anti-mouse immunoglobulin antiserum (Behringwerke, Marburg/Lahn, West Germany) at a final dilution of 1:160 in a mixture of spleen cells, sheep erythrocytes (SRBC), and complement. Numbers of PFC per spleen are expressed as mean values \pm standard error obtained from five or more mice. Group comparisons were made using Student's *t* test.

Preparation of spleen cells. A method for cell preparation described by Lindholm et al. (18) was used. The spleens were passed through a stainless-steel mesh into medium (RPMI 1640) and adjusted to the desired concentration before being tested in the PFC assay or inoculated intravenously into recipient mice.

X-irradiation of cells. Cells were prepared as described above and suspended in RPMI 1640 in petri dishes. The cells were subjected to irradiation using a dose rate of 200 rads/min.

Lymphocyte stimulation. Thymus or spleen cells were suspended in RPMI medium containing 10% human serum, L-glutamine, penicillin, and streptomycin. The cells were cultured in microplate wells, at a volume of 200 μ l and a cell concentration of 5×10^6 /ml, in an atmosphere of 90% N₂, 5% CO₂, and 5% O₂. After addition of concanavalin A (Miles-Yeda,

Rehovot, Israel), the cells were cultured for 3 days. Thereafter 25 μ l of a [14 C]thymidine solution with an activity of 2 μ Ci/ml was added to each well, and thymidine incorporation was determined after another 18 h.

RESULTS

Effect of IF on the antibody response to SRBC in newborn mice. Newborn (less than 1-day-old) DBA/1 \times C57Bl/6 mice were injected with varying doses of IF. After 5 days each mouse received 10^8 SRBC intraperitoneally, and direct and indirect PFC were determined after another 4 days. Mice injected with 128 U or less of IF developed antibody responses as shown in Fig. 1. High doses of IF completely suppressed the response. Low doses (12.8 U) had a stimulatory effect on the direct as well as indirect PFC response. In control experiments, saline, mock IF, or heat-treated (60°C, 1 h) IF had no suppressive or enhancing effect on the antibody response. When the same experiment was performed on 2-week-old instead of newborn mice, no significant enhancing or suppressive effects were obtained.

In other experiments, newborn DBA/1 \times C57Bl/6 mice received daily doses of IF from birth. After 10 days, antigenic stimulation with 10^8 SRBC was performed, and direct and indirect PFC were determined 5 days later. It was found that daily doses of 64 and 640 U of IF enhanced the antibody response, whereas 6.4 U had no evident effect (Fig. 2). A similar enhancement was observed when SRBC were injected 5 days but not 15 days after birth.

Effect of IF on the antibody response to SRBC in adult mice. DBA/1 \times C57Bl/6 mice 4 to 5 weeks old were injected with varying doses of IF at varying times in relation to antigenic stimulation with 10^8 SRBC. Direct and indirect PFC were in all instances determined 5 days after antigenic stimulation. Administration of 1,000 U of IF resulted in suppression of the direct (Fig. 3A) as well as indirect PFC response. A 10-U dose of IF had a significant enhancing effect on the indirect PFC (Fig. 3B), but no evident effect on the direct PFC response. The most pronounced effects were obtained when IF was injected 1 day before antigen.

Potentiation of "allogeneic effect" by IF. Since the "allogeneic effect" in mice is considered to be due to a T-cell-mediated stimulatory effect on host antibody synthesis (18), it was considered possible to use this model to evaluate the effect of IF on T cells. Two types of experiments were carried out. In the first set of experiments, DBA/1 mice were injected with varying doses of IF. Two days later they were sacrificed, and 2×10^7 spleen cells were injected into F1

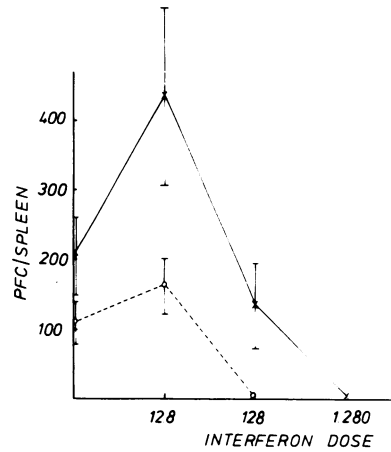


FIG. 1. Effect of varying doses of IF on the antibody response in newborn DBA/1 \times C57Bl/6 mice. Newborn mice were injected with the indicated doses of IF. Five days later, SRBC was injected, and PFC was estimated according to the text. (x) Direct PFC; (o) indirect PFC. Vertical bars in this and the following figures indicate standard error of the mean.

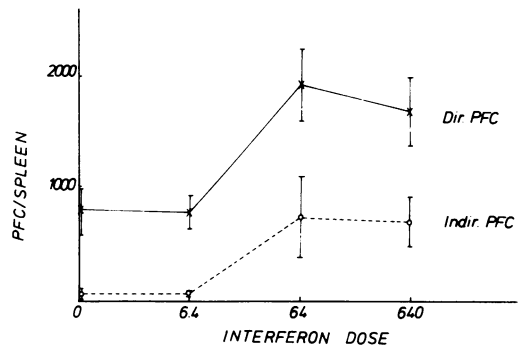


FIG. 2. Effect of varying daily doses of IF on the antibody response in newborn DBA/1 \times C57Bl/6 mice. Newborn mice received daily doses of IF over 10 days. Then SRBC was injected, and PFC was estimated according to the text. (x) Direct PFC; (o) indirect PFC.

hybrid DBA/1 \times C57Bl/6 mice that had been immunized 14 days previously with 10^8 SRBC. Assay of indirect PFC in the spleens of the F1 hybrid mice 5 days after the transfer of the parental cells revealed that spleen cells from the IF-treated DBA/1 mice markedly enhanced the antibody response. This enhancing effect of IF on the stimulatory effect of DBA/1 cells was only evident when spleen cells from mice treated with moderate doses of IF were transferred (Fig. 4). The enhancing effect of 10 and 100 U as well as the suppressive effect of high doses of interferon was statistically significant ($P < 0.01$).

In the other set of experiments, DBA/1 spleen

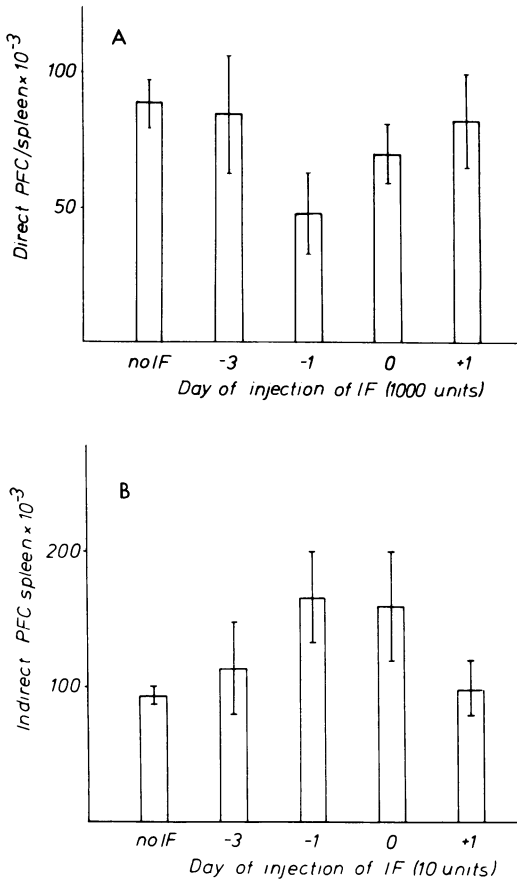


FIG. 3. Influence of IF, injected at different times, on the PFC response in adult mice. Mice were injected with (A) 1,000 U of IF or (B) 10 U of IF at varying times in relation to SRBC injection.

cells were treated *in vitro* with varying doses of IF before transfer to SRBC-primed DBA/1 × C57Bl/6 mice. The cells were incubated with varying dilutions of IF for 2 h and then washed 3 times. Thereafter, 2×10^7 IF-treated cells were transferred to the recipient F1 hybrid mice, which had been injected 14 days earlier with 10^8 SRBC. A statistically significant effect of the IF-treated cells on the indirect PFC response of the F1 hybrid mice similar to that described above was observed also in these experiments (Fig. 4).

To test whether the observed influence of IF on the allogeneic effect was mediated by living cells, experiments using transfer of irradiated (850 rads) parental cells was carried out. An experimental protocol similar to that described above for IF treatment *in vivo* and injection of SRBC and spleen cells was used. IF-treated irradiated parental cells did not augment the antibody response, and no IF-like effect was evoked by mock IF (Fig. 5).

Mitogenic stimulation of IF-treated lymphoid cells. Cultures of spleen or thymus cells were made from adult CBA or CFW mice. IF in varying concentrations was included in the cultures, and an optimal concentration of con-

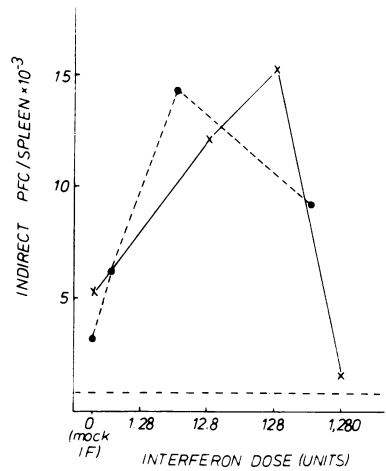


FIG. 4. Potentiation of allogeneic effect by IF. Antibody response in DBA/1 × C57Bl/6 mice injected with DBA/1 spleen cells treated with IF *in vivo* (×) or *in vitro* (●). Number of PFC was determined 19 days after injection of 10^8 SRBC and 5 days after transfer of IF-treated DBA/1 spleen cells. Horizontal line (---) indicates PFC response in mice not injected with parental spleen cells. IF dose was the number of units injected into the donor mice or the number of units per milliliter used for the *in vitro* treatment of the donor cells.

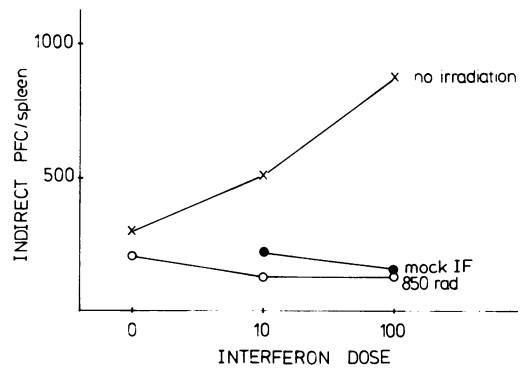


FIG. 5. Abolishment of antibody-potentiating effect of IF-treated parental cells by irradiation. Experimental design was as in Fig. 4 except transfer of parental cells (treated *in vivo* with IF) was performed 3 weeks instead of 2 weeks after immunization of recipient mice. Mock IF was tested in two dilutions, corresponding to the dilution of IF containing 10 and 100 U, respectively. (×) No irradiation of transferred IF-treated parental cells; (○) parental cells irradiated with 850 rads; (●) parental cells treated with mock IF.

canavalin A (10 $\mu\text{g/ml}$) was then added. After 3 days of incubation, [^{14}C]thymidine incorporation in the cells was measured. Low concentrations of IF enhanced mitogenic stimulation of thymus as well as spleen cells in CBA mice, whereas high doses were inhibitory for thymus cells and less stimulatory for spleen cells (Fig. 6).

Similar effects of IF were observed in CFW mice, although the enhancement of deoxyribonucleic acid (DNA) synthesis was more pronounced and was observed even at an IF concentration of 10,000 U/ml. In no instance was any significant inhibitory or stimulatory effect evoked by mock IF.

IF injected into adult mice had significant effects on lymphoid cells, revealed by mitogenic stimulation of thymus and spleen cells 3 days after IF injection. As found in the experiments described above, IF increased the mitogenic response of spleen cells from CBA as well as CFW mice (Fig. 7). In further analogy with these results, the stimulatory effect of IF was significant even after injection of high doses of IF (10,000 U) in CFW mice, whereas CBA spleen cells were inhibited by these doses. In all experiments, the DNA synthetic response of thymus cells was inhibited by high doses of IF (Fig. 7),

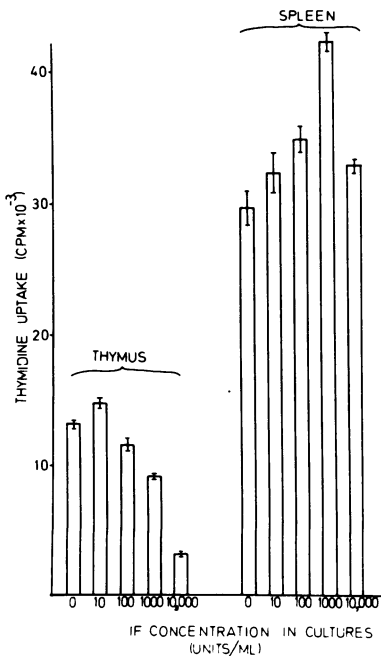


FIG. 6. Influence of IF on DNA synthesis in concanavalin A-stimulated CBA thymus and spleen cells. IF in varying concentrations was added simultaneously with concanavalin A to the cultures, and [^{14}C]thymidine incorporation was measured after 3 days.

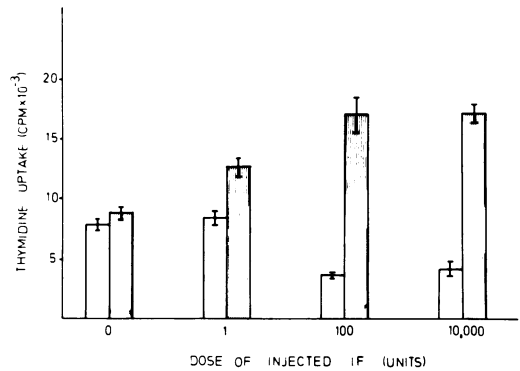


FIG. 7. DNA synthesis in concanavalin A-stimulated thymus and spleen cells from CFW mice injected with varying doses of IF. Cultures were set up 3 days after IF injection. Striped columns indicate thymidine uptake in spleen cell cultures, and open columns indicate uptake in thymus cells.

and no statistically significant enhancement was observed with low doses of IF.

DISCUSSION

IF, which was originally described as an antiviral substance, has attracted renewed interest after the discovery of its diverse effects on cellular functions. Among these effects, those concerning immune responses have been somewhat confusing, since IF has been shown to enhance as well as to suppress antibody responses (2, 3, 7). In the present study we were able to confirm this dual nature of IF function.

It is difficult to exclude the possibility that the activities described both in this paper and in other studies on the biological effects of IF are due to contaminating substances in the preparations used. However, the fact that the effects described in the present report were obtained with very high dilutions of the preparations used and not with mock IF makes it highly probable that IF molecules were responsible for the activities. Confirmatory evidence of this will have to await the results of experiments using pure IF or highly specific antibody to mouse IF.

In adult mice, the most pronounced effect of IF was obtained when it was injected 1 day before antigenic stimulation. No demonstrable effect was observed if IF was given more than 3 days before or more than 1 day after antigen. Since IF has a very rapid turnover (11), these findings suggest that IF acts on stimulated but not on unstimulated immunocompetent cells and also that this action is an early event during cell proliferation. These suggestions are concordant with the findings of Heine and Adler (10) and Weinstein et al. (27). It is also compatible with our findings that IF acts primarily on a

point during the early G1 phase of the fibroblast cell cycle, where the control of DNA synthesis occurs (E. Lundgren, I. Larsson, H. Miörner, and Ö. Strannegård, *J. Gen. Virol.*, in press). Also, in studies of the effect of IF on lymphocyte mitogenesis we have seen no effect of IF pretreatment of lymphoid cells prior to mitogenic stimulation (19a). Thus, all evidence indicates that IF evokes an effect on lymphoid cell function, but only on cells that are stimulated to divide, and primarily during the early regulatory stages of the cell cycle.

Several explanations for the enhancing effect of IF can be provided. It may be that the enhancing and suppressive effects of IF are totally unrelated, such as has been suggested by Killander et al. (15), who found that the expression of antigens on the cell surface is enhanced by IF. It cannot be excluded that an effect of IF on macrophages (12) is responsible for its immunopotentiating effect. Since the enhancing effect of IF on phagocytic cells is evident even at very low concentrations of IF, which would not be supposed to suppress cell division (12), the findings of enhancement at low doses and suppression at high doses of IF would be explainable.

The allogeneic effect studies, however, make another explanation possible. This effect, which is caused by stimulation of B cells by transferred T cells (19), was potentiated by IF in the present study. Thus it seems probable that IF may exert a stimulatory effect on antibody-producing cells via an action on T cells. Evidence that IF has stimulatory as well as inhibitory effects on T cells was obtained in our experiments on mitogenic stimulation of thymus and spleen cells. An immunoenhancing effect, mediated via an action of IF on T cells, could then be exerted by two mechanisms, either by increasing T-cell helper activity or by decreasing T-cell suppressor activity.

The IF-induced augmentation of the allogeneic effect is similar to that obtained after treatment of transferred cells with irradiation or cholera toxin, which has been considered to be due to preferential inactivation of suppressor cells (17, 19). Such cells are also known to be very sensitive to inactivation by cyclic adenosine 3',5'-monophosphate (AMP; 13, 23). The modulating effects of IF on antibody responses found in the present study are similar to those of cholera toxin (17). Like cholera toxin, IF appears to react with ganglioside receptors on cell membranes (1, 25). Cholera toxin is one of the most potent activators of cyclic AMP known. By analogy, it would thus seem possible that the action of IF is mediated by a second messenger, such as a cyclic nucleotide. However, although cyclic AMP appears to potentiate the antiviral effect

of IF (6, 26), there is no evidence that the anti-cellular activity of IF is mediated by, or dependent on, cyclic AMP (27), and in preliminary experiments using concanavalin A-activated suppressor cells we have found no evidence of preferential inactivation of suppressor cells.

The finding that IF augments antibody production particularly in newborn mice may lend support to the idea that IF preferentially inactivates suppressor cells, since suppressor-cell activity appears to be more pronounced in young than in old mice (20). However, the results are equally compatible with the possibility that IF, like cyclic AMP and cyclic AMP-inducing substances, promotes immunological maturation (21, 22). The latter interpretation gains support from the recent finding that prostaglandin E, which raises cyclic AMP levels, is produced in IF-treated cells (28).

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