Brief Definitive Report

INHIBITION OF SUPPRESSOR T LYMPHOCYTES BY MURINE INTERFERON β

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Suppressor T lymphocytes (Ts) have been noted in several animal tumor models (1–3). Adoptive transfer of UV irradiation–induced Ts leads to early development of fibrosarcomas in skin grafts that were exposed to subcarcinogenic doses of UV irradiation before grafting (1). In the P815 mastocytoma and the Meth A fibrosarcoma models, tumor-induced Ts downregulate the concomitant antitumor immunity (2, 3). In these two tumor models, adoptive therapy with immune spleen cells causes regression of established tumors only if Ts function is abrogated in the tumor-bearing host. These data show that Ts can accelerate the development of fibrosarcomas (1), permit the growth of immunogenic tumors in immunocompetent hosts, and inhibit otherwise effective antitumor therapy (2, 3). Inhibition of Ts, therefore, may be a useful adjunct in antitumor immunotherapy. Ts have also been noted in the tolerance to contact allergens (4–6). In these models it is possible to induce, transfer, and assay Ts function in 1–2 wk. This rapidity makes these models particularly suitable for the evaluation of modulators of Ts function.

Cyclophosphamide (CY) is the most extensively studied inhibitor of Ts function. Treatment with CY 1–3 d before tolerization can prevent the generation of Ts (7). However, treatment with CY after sensitization can abrogate immunity (7), and treatment during elicitation can abrogate the skin test reactivity (8). In the adoptive immunotherapy of the Meth A fibrosarcoma, the memory T cell function is not affected by CY, while the function of cytolytic effector T cells is abrogated by CY (9). These data suggest that repeated administration of CY may subtract from the efficacy of the antitumor immunotherapy. Therefore, noncytotoxic inhibitors of Ts function may be more suitable as adjuncts in antitumor immunotherapy.

We studied the effect of purified IFN-β and IFN-α on Ts based on the data of Knop et al. (10). These authors showed that IFN-α/β prepared in C-243 cells induced with Newcastle disease virus inhibited the Ts induced by intravenous administration of dinitrobenzene sulfonic acid ($DNBSO_3$) and dinitrophenylated syngeneic lymphoid cells. Since purified murine IFN-α and IFN-β are available, we wished to determine if IFN-α and IFN-β were required together, or if either IFN-α or IFN-β alone were sufficient to inhibit Ts function. When murine IFN-α/β is prepared from cell lines by induction with a virus, 5–15% of the IFN is α

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and 85–95% is β. Since most of the IFN-α/β is IFN-β, we reasoned that IFN-β alone may be sufficient to inhibit Ts function. The effect of purified IFN-α and IFN-β on the Ts-mediated tolerance generated by epicutaneous antigen overload of dinitrofluorobenzene (DNFB) was measured. Our aim was to identify a selective inhibitor of Ts function. We report our data regarding the inhibition of Ts by IFN-β.

Materials and Methods

Mice. 8–10-wk-old BALB/c ByJ mice were purchased from The Jackson Laboratory, Bar Harbor, ME. Abdominal fur was clipped and the mice were rested for 1 wk before starting an experiment. Six age- and sex-matched mice per group were used in individual experiments.

Sensitization and Elicitation. These were accomplished as described previously (8). Briefly, sensitization was done by applying 15 μl of a 0.5% solution of DNFB (Sigma Chemical Co., St. Louis, MO) in acetone and olive oil (4:1) to the shaved abdominal skin on two consecutive days. Elicitation was done 5 d after the last skin painting. 20 μl of a 0.35% DNFB solution in acetone and olive oil (4:1) was applied to the right earlobe. 24 h later, earlobe thickness was measured by a spring-loaded micrometer (Federal, Providence, RI). The difference between the right and the left earlobe thickness was considered to be the extent of delayed-type hypersensitivity (DTH).

Induction of Tolerance and Transfer of Suppression. This was also done as described previously (8). Ts-mediated tolerance was induced by skin painting of the shaved abdominal skin with 150 μl of a 0.5% DNFB solution on two consecutive days. To transfer suppression, 5 d after the last skin painting, the donors of Ts were sacrificed and 10^8 spleen cells were transferred intravenously to each naive recipient mouse. 24 h later the recipients were sensitized and skin tested as described above.

Murine IFN-α, IFN-β, and Mock IFN/Control Buffer. These were purchased from Lee Biomolecular, San Diego, CA. The manufacturer reports that the IFNs were prepared from L cells induced with the Newcastle disease virus and the mock IFN was prepared from sham-induced L cells. The IFNs were purified chromatographically. Discontinuous gel electrophoresis with 8–18% polyacrylamide gradients showed the IFN-α and IFN-β to have molecular masses of 27–28 kD and 35–36 kD respectively. The α and β preparation contained <0.5% IFN of the opposite species. The IFN activity was quantitated by measuring the protective effect against cytocidal infection of L cells with the encephalomyocarditis virus. The results were normalized to National Institutes of Health reference units. The IFN-α (lot 85090) contained 1.2×10^5 U/ml, IFN-β (lots 83059 and 83001) contained 7.6×10^5 U/ml and 1.8×10^6 U/ml, and the mock IFN/control buffer (lot 81016) contained <4 U/ml. On random testing of crude and purified IFN and mock IFN/control buffer, the amount of endotoxin and LPS were 3–10 ng/ml, as determined by the Limulus assay. The interferons and the mock IFN were diluted in Dulbecco's PBS, and the desired amount of IFN in 0.2 ml was injected intravenously in each mouse via the tail vein.

Statistical Analysis. The student's *t* test was used to compare the DTH reading among the various groups.

Results

Effect of IFN-α and -β on the Generation of Ts-mediated Tolerance. Tolerance to DNFB was generated as described in Materials and Methods. IFN-α and IFN-β were injected at 50–1,000 U/mouse i.v., 3 h after the first of two skin paintings done on two consecutive days. Elicitation was done 5 d later. As shown in Table I, IFN-β at 1,000 U/mouse abrogated the generation of tolerance. IFN-α at the same dosage had a much less pronounced effect.

TABLE I

Inhibition of Ts-mediated Tolerance by IFN-β

Group*	Preparation‡	Treatment§	Increase in earlobe thickness‖
			× 10^{-2} mm
1	Sensitize	—	21.5 ± 1.4¶
2	Tolerize	—	4.1 ± 0.7
3	Tolerize	Buffer	3.6 ± 0.9
4	Tolerize	IFN-β, 50 U	2.6 ± 2.0
5	Tolerize	IFN-β, 500 U	8.4 ± 0.9
6	Tolerize	IFN-β, 1,000 U	19.0 ± 0.7¶,**
7	Tolerize	IFN-α, 50 U	4.8 ± 1.6
8	Tolerize	IFN-α, 500 U	5.8 ± 1.1
9	Tolerize	IFN-α, 1,000 U	11.2 ± 0.8

* Six mice per group.
‡ Sensitization done by painting the shaved abdominal skin with 15 μl of a 0.5% DNFB solution on days 1 and 2. Tolerization done by skin painting with 150 μl of a 0.5% DNFB solution on days 1 and 2.
§ IFN, at indicated doses, or buffer in 0.2 ml of D-PBS intravenously 3 h after the first of two skin paintings.
‖ Skin testing done by applying 20 μl of a 0.35% DNFB solution to the right earlobe on day 7. Increase in earlobe thickness measured on day 8, and expressed as the mean ± SEM for each group.
¶ Significantly different ($p < 0.05$) as compared with groups 2–5, and 7–9.
** No significant difference between groups 1 and 6.

TABLE II

Inhibition of the Transfer of Suppression by IFN-β

Group*	10^8 Spleen cells from tolerized mice‡	Treatment§	Increase in earlobe thickness‖
			× 10^{-2} mm
1	−	—	26.3 ± 1.2¶,**
2	+	—	6.0 ± 1.0
3	+	Buffer	5.6 ± 1.6
4	+	IFN-β, 50 U	7.6 ± 1.6
5	+	IFN-β, 500 U	11.2 ± 1.6
6	+	IFN-β, 1,000 U	24.8 ± 1.2¶,**

All six groups were sensitized by painting with 15 μl of a 0.5% DNFB solution on the shaved abdominal skin on two consecutive days starting 24 h after the transfer of spleen cells.
* Six mice per group.
‡ Spleen cells from mice tolerized to DNFB 5 d previously.
§ IFN, at indicated doses, or buffer in 0.2 ml D-PBS intravenously 3 h after the transfer of spleen cells.
‖ Skin testing done by applying 20 μl of a 0.35% DNFB solution to the right earlobe 5 d after the second of two sensitizations. Increase in earlobe thickness measured 24 h later, and expressed as mean ± SEM for each group.
¶ Significantly different ($p < 0.05$) as compared with groups 2–4.
** No significant difference between groups 1 and 5.

Because our aim was to identify an inhibitor of Ts function, further experiments were done with IFN-β.

Effect of IFN-β on the Transfer of Suppression by Ts. Tolerance to DNFB was generated in the donors of Ts as described in Materials and Methods. 5 d later, 10^8 spleen cells from these tolerized mice were intravenously transferred to each naive mouse. 3 h later, IFN-β was injected intravenously at 50–1,000 U/recipient mouse. Sensitization was done over the next 2 d, and elicitation was done 5 d after completion of sensitization. As shown in Table II, IFN-β at 1,000 U/mouse

TABLE III

Lack of Effect of IFN-β on Sensitization and Elicitation

Group*	Treatment during sensitization‡	Treatment during elicitation§	Increase in earlobe thickness‖
			$\times 10^{-2}$ mm
1	—	—	26.3 ± 1.2
2	IFN-β, 1,000 U	—	24.6 ± 1.8
3	IFN-β, 10,000 U	—	28.0 ± 2.4
4	—	IFN-β, 1,000 U	22.8 ± 0.9
5	—	IFN-β, 10,000 U	28.0 ± 2.5

* Six mice per group.

‡ IFN in 0.2 ml of D-PBS injected intravenously 3 h after the first of two daily paintings with 15 μl of a 0.5% DNFB solution on the shaved abdominal skin.

§ IFN, at indicated doses, in 0.2 ml of D-PBS injected intravenously 3 h after applying 20 μl of a 0.35% DNFB solution to the right earlobe 5 d after the second sensitization.

‖ Increase in earlobe thickness measured 24 h after applying the skin test, and expressed as mean ± SEM for each group. No significant differences among groups 1–5.

TABLE IV

Effect of IFN-β on Established Tolerance

Group*	Days 1–2‡	Days 7–8§	Increase in earlobe thickness‖
			$\times 10^{-2}$ mm
1	—	Sensitize	25.8 ± 2.4¶
2	—	Tolerize	2.0 ± 0.5
3	Tolerize	Sensitize, buffer**	3.1 ± 0.6
4	Tolerize	Sensitize, IFN-β, 100 U	4.1 ± 0.6
5	Tolerize	Sensitize, IFN-β, 500 U	4.2 ± 1.0
6	Tolerize	Sensitize, IFN-β, 1,000 U	4.2 ± 0.6

* Six mice per group.

‡ Tolerization done on days 1 and 2 by painting 150 μl of a 0.5% DNFB solution on the shaved abdominal skin for groups 3–6.

§ Sensitization done on days 7 and 8 by painting 15 μl of 0.5% DNFB solution on the shaved dorsum of the torso for groups 1, 3–6. Tolerization done on days 7 and 8 by painting 150 μl of 0.5% DNFB solution on the shaved dorsum of the torso for group 2.

‖ Skin testing done on day 13 by applying 20 μl of a 0.35% DNFB solution to the right earlobe. Increase in earlobe thickness measured 24 h after applying the skin test, and expressed as mean ± SEM for each group. No significant difference among groups 2–6.

¶ Significantly different ($p < 0.05$) as compared with groups 2–6.

** IFN-β, at indicated doses, or mock IFN/control buffer, at indicated doses, in 0.2 ml of D-PBS injected intravenously in groups 3–6 3 h after the first of two sensitizations.

completely abrogated the transfer of suppression by Ts. IFN-β at 500 U/mouse abrogated the transfer of suppression to a lesser extent.

Effect of IFN-β on the Sensitization and Elicitation. Sensitization and elicitation were done as described in Materials and Methods. IFN-β was injected 1,000 and 10,000 U/mouse, i.v., either 3 h after the first of two sensitizations or 3 h after elicitation. As shown in Table III, IFN-β up to 10,000 U/mouse had no adverse effect on sensitization and elicitation.

Effect of IFN-β on Established Tolerance. Tolerance to DNFB was generated as described in Materials and Methods. 5 d after the second skin painting, the mice were sensitized by skin painting with 15 μl of a 0.5% DNFB solution on the shaved dorsal aspect of the torso, on two consecutive days. IFN-β was injected at 1,000–10,000 U/mouse i.v. 3 h after the first sensitization. Elicitation was done 5 d after the second of two sensitizations. As shown in Table IV IFN-β in the doses tested had no effect on the persistence of tolerance.

Discussion

The regulatory and suppressive mechanisms that control the induction, expression, and duration of contact sensitivity to DNFB have been studied extensively (11). Skin painting with high doses of DNFB leads to the generation of antigen-specific Ts that are detectable by day 3, peak by day 5, and are no longer detectable by day 11 (8, 11). Treatment of the host with CY before antigen overload leads to the generation of sensitization. Testing of Ts-inhibitory agents in this setting, therefore, permits the demonstration of sensitization in the recipient without having to do cell transfer experiments. It is for this reason that epicutaneous antigen overload with DNFB was chosen herein as the method for inducing the Ts-mediated tolerance.

The data presented show that IFN-β alone at 1,000 U/mouse i.v. was sufficient to inhibit the generation of Ts-mediated tolerance (Table I, group 6), and to abrogate the transfer of suppression of sensitization by Ts (Table II, group 6). Comparable doses of IFN-α had a less pronounced inhibitory effect on the generation of tolerance (Table 1, group 9). It is conceivable that higher doses of IFN-α may abrogate Ts function. The data also show that a 10-fold higher dose of IFN-β had no adverse effect on sensitization or elicitation (Table III, groups 3 and 5). This is in contrast to CY, which can adversely affect sensitization (7) and elicitation (8). Thus, IFN-β seems to be a suitable agent for evaluation as an adjunct in the immunotherapy of established Ts-inducing tumors.

The mechanism of the inhibitory effect of IFN-β remains undefined. Since IFN-β is administered in vivo, it is possible that it may be acting directly on Ts or indirectly via its effect on the antigen-presenting cells (APCs). The latter seems unlikely, since incubation of spleen cells from tolerized mice with IFN-α/β in vitro, before transfer into naive recipients, is sufficient to abrogate the transfer of suppression (10). The antiproliferative effect of IFN is mediated by (2′,5′)-oligoadenylate (oligoA) (12). However, since it is difficult to enrich antigen-specific Ts, it has not been possible to determine if IFN-β induces oligoA to a greater extent in Ts as compared with other T cells.

The concentration of endotoxin and LPS, as determined by the Limulus assay in the crude and purified IFN preparations, and in the mock IFN/control buffer is 3–10 ng/ml. Since the mock IFN had no effect, and since IFN-α had minimal effect on the generation of the Ts-mediated tolerance, it seems unlikely that the contaminating endotoxin or LPS were responsible for the observed effects.

We have extended the observation of Knop et al. (10) in two respects. First, IFN-β alone, as opposed to IFN-α/β, was shown to be sufficient for inhibition of Ts. Second, administration of IFN-β at the time of tolerizing exposure to DNFB was shown to lead to sensitization. Having shown that IFN-β abrogates Ts function without adversely affecting sensitization or elicitation with DNFB, it would be important to test its effect on Ts function in a tumor model such as the P815 mastocytoma or the meth A fibrosarcoma.

Summary

We have presented data showing that IFN-β at 1,000 U/mouse i.v. inhibits the generation of Ts-mediated tolerance to dinitrofluorobenzene (DNFB) and abrogates the transfer of suppression by Ts. We have also presented data showing

that IFN-β up to 10,000 U/mouse i.v. has no adverse effect on sensitization and elicitation. IFN-β appears to be a suitable agent for evaluation as an adjunct in the immunotherapy of Ts inducing tumors.

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