Effect of Interferon on the Structural Organization of Rat Thymus

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Changes in the thymus of male Wistar rats were studied under a light microscope at various times after intranasal administration of α-interferon. The relative mass of the organ, the cortex volume, the total number of cells, the number of small and medium lymphocytes, and the number of mitoses decrease 14 days after interferon administration. At the same time, the number of macrophages, neutrophils, mature plasmacytes, eosinophils, and erythrocytes increases, and mast cells appear. Thus, α-interferon probably suppresses the formation of T cells, facilitates allergization of the organism, and increases the permeability of the vascular endothelium.

Key Words: *interferon; thymus; cells*

At the present time, interferons (IFN) are used not only in the treatment and prevention of viral infections but also as preparations with pronounced immunomodulatory and antitumor activities.

The addition of IFN to a culture of T cells considerably boosts the proliferative activity [8], thus enhancing the proliferative response to mitogens and other biologically active substances [6]. However, there is evidence that even low doses of α-IFN inhibit T-cell proliferation [5], while an increase in the dose is directly proportional to the decrease in lymphocyte number in the peripheral blood and the thoracic duct [9]. The effect of IFN probably depends on the dose and route of administration [3]. There may be a correlation between the influence of the preparation *in vitro* and *in vivo* [4].

MATERIALS AND METHODS

Experiments were performed on male Wistar rats. An aqueous solution of reaferon (a recombinant human α_2-IFN, 50 U/ml) was administered intranasally (2 drops) 4 times a day during a 5-day period. Control animals received distilled water.

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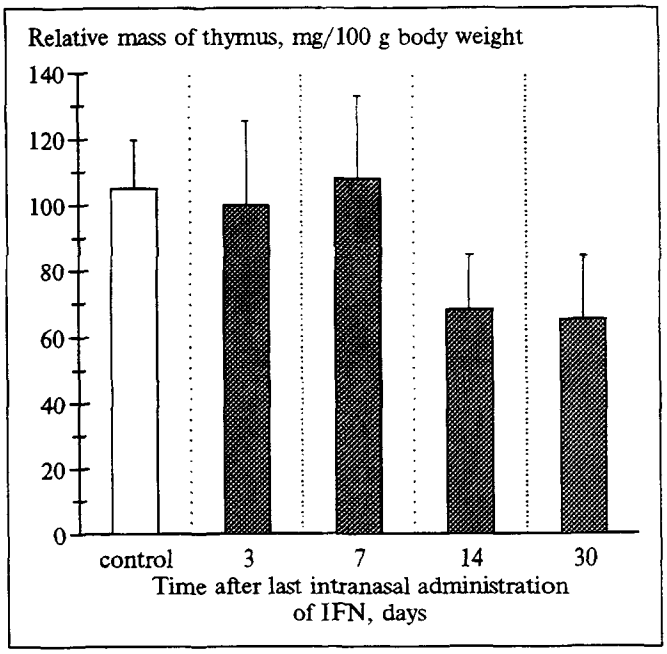

Fig. 1. Changes in the relative mass of the thymus. Abscissa: time after last intranasal administration of IFN, days; ordinate: relative mass of thymus (mg/100 g body weight).

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For morphological studies the thymus was taken on days 3, 7, 14, and 30 after the last IFN administration. Thirty animals were used per time point. The samples were fixed in 4% paraformaldehyde in phosphate buffer, dehydrated in ascending grades of ethanol, treated with xylol, and embedded in paraffin. Sections (5 μ thick) were stained with hematoxylin and eosin, azure II-eosin, and after Van Gieson. A 32-fold magnification was sufficient for observation of the entire section area and reliable identification of the studied structures. The cells of plasmacyte origin were determined according to the published classification [1]. Test systems were chosen according to the recommendations [12]. The differences between the mean values were considered significant at $p<0.05$ (Student's t test).

RESULTS

The changes in microanatomical organization of the thymus after intranasal administration of IFN are illustrated in Figs. 1 and 2, and the data on the cytoarchitectonic dynamics are summarized in Table 1.

The involution of the thymus due to a decrease in the volume of the medulla and the number of lymphocytes, which started on the 14th day, suggests inhibition of T-cell proliferative activity. This inhibition probably makes it possible to obtain animals with lowered thymic function which can be used as a model of T-cell immunodeficiency. The use of IFN for this purpose may be economically advantageous, since it is more cost-effective than the conventional surgery. On the basis of the IFN-induced thymic alterations it can be expected that reaferon will be effective in the treatment of T cell leukemias.

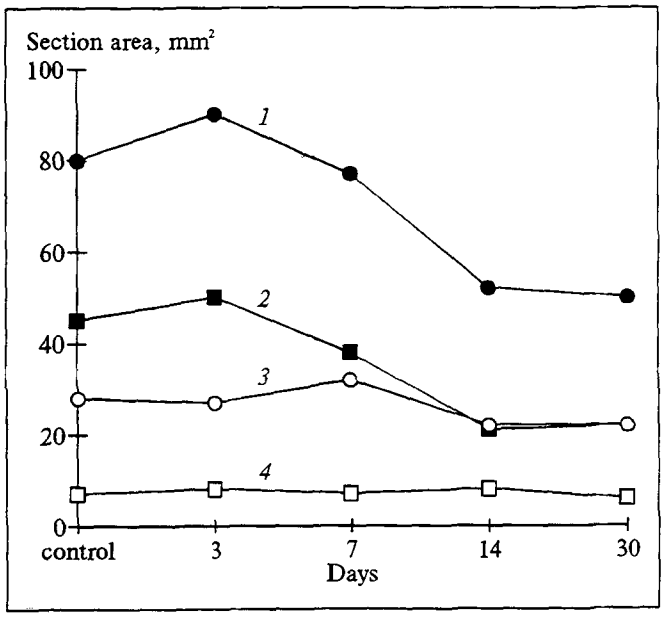

Fig. 2. Changes in the cross section of rat thymus zones. Abscissa: days after last administration of IFN. The asterisk indicates statistically significant difference from the control ($p<0.05$). *1*) total area of thymic section; *2*) area of cortical section; *3*) area of medullar section; *4*) are of capsule and septal section.

It is unclear just what caused the accelerated differentiation and the appearance of plasmacytes in the cortex (Fig. 3): whether it is a direct influence of IFN promoting the proliferation of B cells

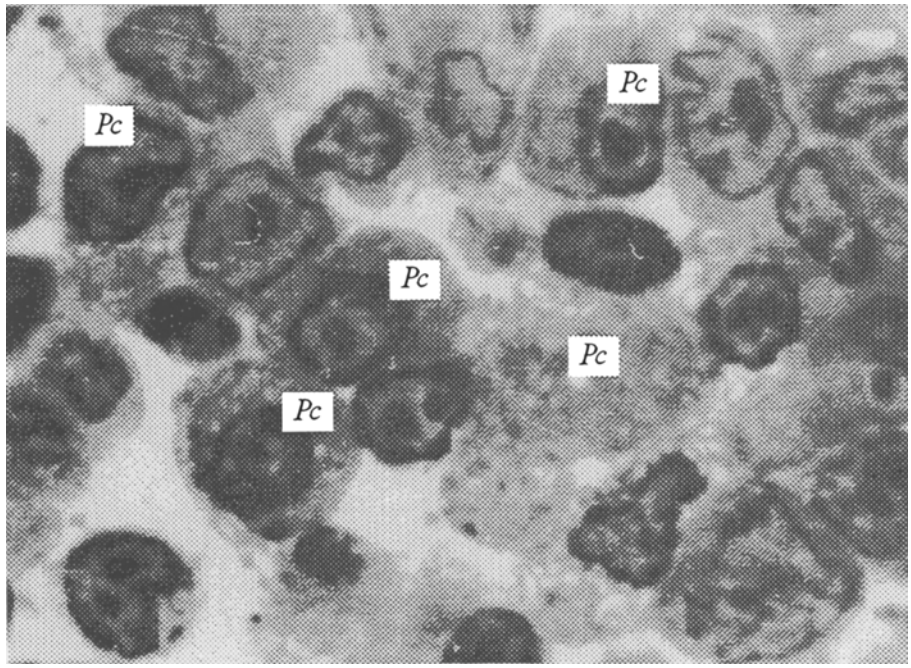

Fig. 3. Plasmacytes in rat thymus cortex 14 days after last intranasal administration of IFN. *Pc*: plasmacytes. Azure–eosin. Oc. 10, obj. 125.

TABLE 1. Changes in Cellular Composition (Cell Number×1000 per Zone Section) of Thymic Lymphoid Parenchyma at Different Times after Intranasal Administration of IFN ($M \pm m$)

Cells	Control	Time after last administration of IFN, days			
		3	7	14	30
		Cortex			
Small lymphocytes	894±56	1195±124*	686±49*	138±12*	93.6±5.9*
Medium lymphocytes	52.7±4.8	32.6±3.1*	12.7±1*	10.8±0.8*	8.4±0.7*
Immunoblasts	24.4±2.2	19.2±2	8.4±0.7*	10.7±0.8*	15.4±1.4*
Plasmoblasts	–	–	–	1.2±0.1*	0.8±0.1*
Immature plasmocytes	–	–	–	2.4±0.2*	0.9±0.2*
Mature plasmocytes–	1.7±0.2*	2.6±1.9*			
Mott cells	–	–	–	0.1±0.01*	0.08±0.01*
Stellate epitheliocytes	127±11	119±10.4	157±12	127±14	138±12
Monocytes	15.3±1.3	9.7±0.8*	5.6±0.4*	7.7±0.6*	14.2±1.5
Macrophages	29.8±2.7	41.6±4.1*	67.4±4.9*	94.3±8.2*	57.4±5.5*
Neutrophils	–	5.2±0.4*	12.4±1.1*	1±0.1*	1.7±0.2*
Eosinophils	0.5±0.1	4.7±0.5*	3.2±0.2*	1.2±0.1*	–
Mast cells	–	–	5.6±0.4*	0.9±0.1*	–
Degenerating cells	4.3±0.3	6.7±0.4*	8.8±0.7*	4.9±0.4	5.6±0.5*
Erythrocytes	–	2.1±0.2*	0.5±0.1*	0.8±0.1*	0.9±0.2*
Mitoses	84.7±5.9	44.7±6.5*	52.6±4.2*	39.9±4.2*	57.4±5.2*
Total	1233±113	1480±126	1020±89	443±41*	397±36*
		Medulla			
Small lymphocytes	297±24	208±19*	129±10*	26.5±2.3*	98.9±7.3*
Medium lymphocytes	52.6±4.9	47.2±4.4	64.2±6.5	22.4±1.2*	62.7±5.4
Immunoblasts	27.6±3.1	49.3±5.1*	53.6±6.6*	19.7±3	39.9±3.2*
Plasmoblasts	14.9±1.5	27.5±2.4*	39.2±3.1*	10.7±1.1*	17.3±1.2
Immature lymphocytes	2.2±0.2	5.2±0.5*	0.1±0.01*	0.1±0.01*	0.9±0.1*
Mature lymphocytes	0.9±0.1	1.5±0.1*	0.5±0.1*	1.9±0.2*	2.5±0.2*
Mott cells	–	–	0.1±0.01*	0.2±0.02*	0.1±0.01*
Stellate epitheliocytes	67.4±5.2	84.3±7.2	94.7±6.9*	67.5±6.2	61.4±6.2
Monocytes	5.7±0.5	2.3±0.2*	3.3±0.3*	10.2±1.3*	4.7±0.5
Macrophages	10.3±0.9	15.9±1.6*	21.4±2.2*	15.7±1.3*	21.3±2.2*
Neutrophils	1.7±0.2	5.9±0.6*	8.4±0.7*	3.8±0.4*	1.4±0.1
Eosinophils	0.9±0.1	6.6±0.7*	7.2±0.6*	2.7±0.3*	–
Mast cells	–	2.7±0.3*	4.4±0.5*	1.4±0.1*	–
Degenerating cells	6.3±0.5	7.4±0.6	9.2±0.8*	5.2±0.5	4.7±0.4*
Erythrocytes	0.8±0.1	2.8±0.2*	4.3±0.4*	0.9±0.1	0.9±0.1
Mitoses	67.3±6.2	37.6±4*	42.7±4.7*	41.7±3.7*	92.7±8.3*
Hassall's corpuscles	1.2±0.1	1.5±0.2	1±0.1	2.4±0.2*	2.6±0.2*
Total	557±39	506±42	483±42	233±19*	412±36*

Note. Values significantly different from the control are indicated with an asterisk ($p<0.05$).

and their differentiation to plasmacytes or the formation of anti-IFN antibodies.

The preparation has been reported to activate macrophage migration but not to directly stimulate neutrophil chemotaxis [11]. However, IFN can induce synthesis of other cytokines, which in turn can stimulate lymphocyte secretion of the chemotaxis factor for neutrophils [11], possibly leading to an increase in the phagocyte content of the lymphoid organs.

Peripheral blood eosinophilia occurs for all routes of reaferon administration and strongly correlates with the dose [2]. On the other hand, it has been demonstrated that even microdoses of γ-IFN induce degranulation of mast cells [10]. The increase in the number of eosinophils and mast cells is evidently due to the antigenic activity of the preparation.

It was shown *in vitro* that the binding of T cells to mast cells increases considerably after treatment of the latter with α- or γ-IFN [7,13]. This may activate lymphocyte migration to the perivascular space and increase vascular permeability or even damage the capillary endothelium after the administration of IFN [10].

Presumably, under the influence of IFN the number of cells decreases not only due to their reduced secretion by the bone marrow but also to lowered proliferative activity *in situ*. The reduced

number of mitoses in the thymus occurring before the decrease in the total cell number may confirm this assumption.

The marked changes occurring in the thymus cannot be attributed solely to the effect of IFN delivered into the bloodstream. Interferon may find its way into regional peripheral nodes, where it activates some cells. A portion of these cells then enters the bloodstream and other organs. It cannot be excluded that most of the observed changes are induced by substances secreted by IFN-activated cells.

Our results indicate that IFN administration may lead to the involution of the thymus, allergization of the organism, and an increase in vascular permeability.

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Evaluation of Microsomal and Mitochondrial Oxidation in Rat Liver in Tetracycline-Induced Hepatosis

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A satistically significant decrease in the content of cytochromes P-450 and b_5 and in the activity of aniline hydroxylase and p-nitroanisole demethylase occurs in rat liver microsomes during the development of experimental acute fatty hepatosis develoing within a 24-h period after intraperitoneal administration of 125 mg/kg tetracycline hydrochloride. Under these experimental conditions tetracycline hydrochloride elicits only an insignificant disintegrating effect on oxidative phosphorylation in liver mitochondria.

Key Words: *tetracycline-induced fatty hepatosis; microsomal and mitochondrial oxidation; oxidative phosphorylation*

The toxic effect of tetracycline (TC) at the molecular and submolecular levels is due to its high membrane tropism and chelating properties. Binding of TC by mitochondria and chelation of Mg^{2+}, which plays an important role in oxidative phosphorylation, are thought to be implicated in TC toxicity [8,11]. Depending on dose and duration of the administration period, TC elicits either a disintegrating or an inhibitory effect on cell respiration [6,15].

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