The Correlation between Sympathetic Denervation and Interleukin-1 Production by Alveolar Macrophages during Chemical Carcinogenesis in the Lungs

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Considerable attention has been focused recently on the role macrophages play in the regulation of both sympathetic neuron activity and the regeneration of nervous tissue [8]. It has been shown that macrophages realize their effect by the production of interleukin-1 (IL-1) [7-9]. It is agreed that IL-1 is responsible for increasing nerve growth factor (NGF) production by fibroblasts [10] and Schwann cells [6]. Nerve growth factor is an obligatory trophic factor for sympathetic neurons. In postnatal ontogenesis NGF enhances the growth and regeneration of adrenergic axons and prevent effects of sympathectomy [11]. *In vivo* the direct effect of NFG on immune cells is the opposite of the effect of sympathectomy [3], and the immune response is strengthened. However, the effects of the sympathetic nervous system on IL-1 production by macrophages have not been adequately investigated.

Alterations of sympathicoadrenal system in carcinogenesis are known to manifest themselves in a reduction of adrenoreceptor affinity [1], sympathetic denervation of various organs [2], and persistent elevation of sympathetic tonicity. At the same time, various disturbances of immunocellular activity, including macrophage IL-1 production, are observed [13].

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Thus, it is important to know whether the changes in IL-1 production in carcinogenesis are associated with the pathology of the sympathetic innervation. The aim of this study was to establish the correlation between the morphofunctional alterations of sympathetic innervation of the lungs with IL-1 production by alveolar macrophages during urethane-induced carcinogenesis.

MATERIALS AND METHODS

The experiments were carried out in summer on 45 2.5-month-old male BALB/c Sto mice weighing 20-21 g. The mice of the experimental group received i.p. a 10% solution of urethane (Reakhim) in medium 199 in a dose of 0.7 mg/g eight times during one month. The control animals received an equal volume of medium 199 i.p. at the same times. The mice were killed by neck dislocation 1, 2, 3, and 4 months after the start of urethane treatment. Some of the experimental animals with tumors which were killed in the third month received during the last four weeks NGF i.v. 325 ng/g per injection according to a scheme described elsewhere [3] (NGF was made available by V.N. Kalyunov, Institute of Physiology, Academy of Science of Belarus).

The density of sympathetic innervation, the catecholamine content in the lung parenchyma, and

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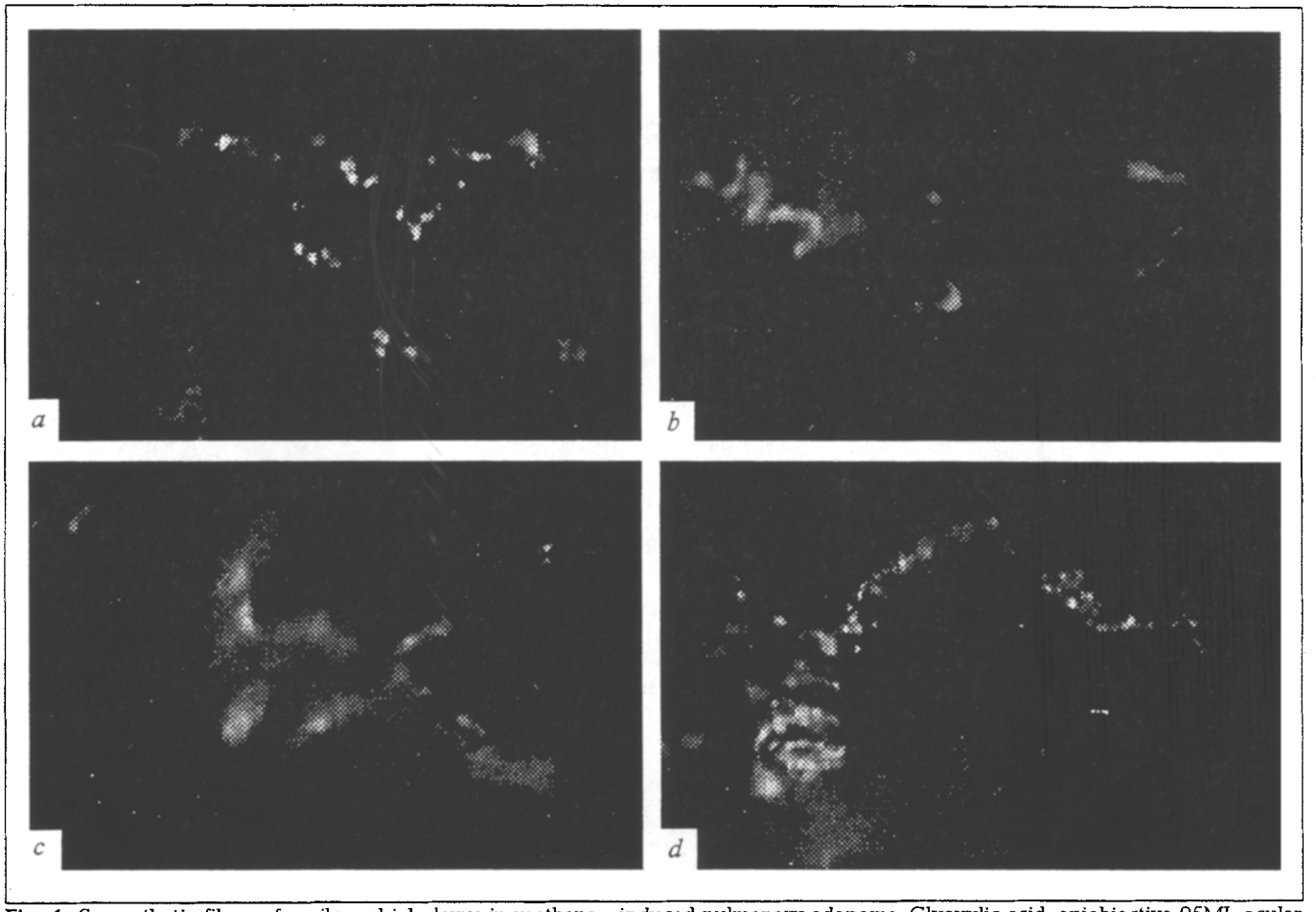

Fig. 1. Sympathetic fibers of peribronchial plexus in urethane–induced pulmonary adenoma. Glyoxylic acid, epiobjective 95MI, ocular 10. *a*) intact fibers; *b*, *c*) nerve fibers with features of hyperfunction; *d*) after a one–month course of NGF.

IL-1 production by alveolar macrophages were examined for all groups of animals under investigation.

The density of sympathetic innervation was computed on cryostat sections 25 μ thick. The sections were cut in the plane parallel to the mediastinal surface of the lung lobe crossing the lobar bronchus bifurcation. Nerve fibers were stained with glyoxylic acid [15].

The slides were examined under a LYuMAM luminescence microscope with S3S21-2, SS15, and FS1-2 exciting filters, epiobjective 95MI, ocular ×7. The photometry procedure was performed with an FMEL-1A spectrofluorometric attachment with interference filter № 6. A Stropus point grid was used to determine the density of sympathetic fibers. The whole surface of the slide was examined and the result was expressed as the number of intersections per 100 visual fields.

Catecholamine content was measured with epiobjective 21, ocular ×10, probe diameter 0.5 at 25 randomly chosen points. The intensity of fluorescence was expressed in conventional units (c.u.).

Alveolar macrophages obtained from a bronchoalveolar flush were washed three times with Eagle's medium and concentrated by adhesion to plastic. Adherent cells in a concentration of 10^6 cells/ml were incubated in 24-well plates (Nunclon) for 18 h in 5% CO_2 incubators (37°C).

Five percent embryonal calf serum, 2 mmol L-glutamine (Sigma), and 80 μg/ml gentamicin were added to the culture medium (RPMI-1640) for macrophage cultivation. *E. coli* lipopolysaccharide (LPS) 0111:B4 (Difco) was used as macrophage stimulator in a concentration of 10 μg/ml. The activity of IL-1 was tested in the supernatant by the

TABLE 1. Density (Number of Intersections per 100 Visual Fields) of Sympathetic Innervation of Lungs in BALB/c Mice under Urethane–Induced Carcinogenesis ($M \pm m$)

Time from beginning of urethane injection, months	Agent		
	Urethane	Medium 199	Urethane and NGF
1	49.8±9.5	34.5±3.3*	–
2	37.7±4.1	48.3±6.4*	–
3	11.6±1.8	29.2±5.8*	43.4±5.4*
4	19.4±3.2	46.5±8.3*	–

Note: here and in Table 2 an asterisk signifies that the difference from the control (medium 199) is significant ($p<0.05$).

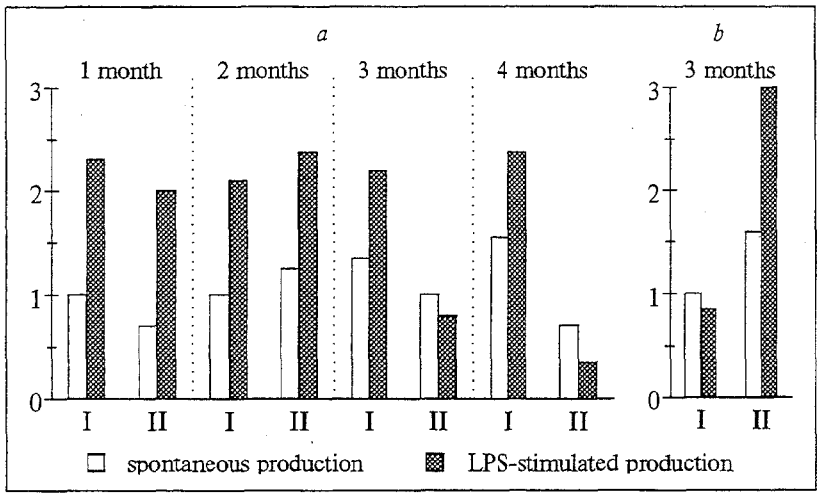

Fig. 2. Dynamics of IL-1 production by alveolar macrophages in urethane-induced pulmonary adenoma. Abscissa: time from beginning of urethane injection (months); ordinate: IL-1 activity in supernatant (in EI). White columns: spontaneous production, shaded columns: LPS-stimulated production. *a*: I) control group, II) experimental group; *b*: I) experimental group in second month of carcinogenesis; II) same as I, with prior one-month course of NGF.

routine method of measuring the proliferation response of mice thymocytes [12] and expressed as the effective index (EI):

$$EI = \frac{\text{number of cpm in ConA-induced culture of thymocytes with supernatant of cells}}{\text{number of cpm in ConA-induced culture of thymocytes without supernatant}}$$

Statistical analysis of the data was performed using the nonparametric Wilcoxson-Mann-Whitney *U* test.

RESULTS

It has been shown previously [14] and was reconfirmed here that urethane injection towards the end of the third month results in histologically and visually identifiable carcinoma nodes in the lungs of BALB/c mice. As this took place, the adrenergic nerve fiber density increased significantly in comparison with the control right at the end of one month of carcinogen treatment and then steadily decreased until the end of the third month of treatment. Thus, the density of adrenergic innervation was already significantly decreased after two months of treatment and during the third month decreased by one half. During the fourth month this ratio was preserved (Table 1).

In the third month of carcinogenesis a functional heterogeneity of the sympathetic nerve fibers was observed together with a decreased density of adrenergic innervation. While a majority of the neurons had a pronounced varicose structure (Fig. 1, *a*), as was the case in the controls, some of them brightly fluoresced without any varicosities (Fig. 1, *b*) and had blurred outlines due to catecholamine diffusion (Fig. 1, *c*). The hyperfunction of some adrenergic fibers may be a compensatory mechanism due to the process of sympathetic denervation, as has already been reported in the literature [5].

The dynamics of the catecholamine content in the lung parenchyma was of the opposite nature. Decreased at the end of the first month, the content did not differ significantly in the experimental and control groups towards the second month and by the third month was 1.3 times higher than in the control. Over the fourth month the catecholamine concentration increased until it became 1.6 times as high as in the control (Table 2).

The increase of catecholamines in the lung parenchyma during carcinogenesis may be related to the hyperfunction of some nerve fibers, impaired catecholamine reception and metabolism, decreased neurotransmitter reuptake, and synchronous activation of the adrenal medulla [1].

From the end of the third month of carcinogenesis IL-1 production by alveolar macrophages stimulated with LPS fell markedly while the spontaneous level of production was preserved. By the end of the fourth month both spontaneous and stimulated production were decreased (Fig. 2, *a*).

The treatment with NGF prevented the processes of desympathization (Fig. 1, *d*, Table 1) and the reduction of IL-1 production (Fig. 2, *b*) but the catecholamine content in the lung tissue was not affected (Table 2).

The findings do not allow us to deduce whether the described phenomenon primarily arises from the direct effect of NGF on the immune cells or whether this plays a protective role for the sympathetic neurons. However, it should be taken into account that many of the sympathetic effects relate to the changes of the local synthesis of NGF by axons. Nerve growth factor is also constitutively produced in various tissues throughout ontogenesis.

TABLE 2. Catecholamine Content (Conventional Units of Luminescence) in Lung Parenchyma of BALB/c Mice under Urethane-Induced Carcinogenesis ($M \pm m$)

Time from beginning of urethane injection, months	Agent		
	Urethane	Medium 199	Urethane and NGF
1	11.7±0.3	13.0±0.8*	–
2	8.3±0.2	8.0±0.5	–
3	13.8±0.4	10.3±0.2*	14.2±0.53
4	14.7±0.2	9.3±0.3*	–

Hence, the results presented suggest that the sympathetic nervous system can modulate IL-1 production by the macrophages through the release of NGF, affecting both the immune cells and adrenergic neurons. Disturbances in these regulatory processes probably take place during carcinogenesis.

REFERENCES

1. N. M. Gogitidze and M. D. Gedevanishvili, *Eksp. Onkologiya*, **12**, № 5, 29 (1990).
2. V. D. Dyshlovoi, in: *Changes in the Organism in Cancer* [in Russian], Arkhangel'sk (1967), p.75.
3. V. N. Kalyunov, *Nerve Growth Factor* [in Russian], Minsk (1984).
4. D. N. Mayanskii and D. D. Tsyrendorzhiev, *Pat. Fiziol.*, № 4, 44 (1990).
5. V. N. Yarygin, I. M. Rodionov, and L. M. Giber, *Tsitologiya*, **12**, № 6, 745 (1976).
6. K. Bergsteinsdottir, A. Kingston, R. Mirsky, *et al., J. Neuroimmunol.*, **34**, No 1, 15 (1991).
7. V. Guenard, C. A. Dinarello, P. J. Weston, *et al., J. Neurosci. Res.*, **29**, № 3, 369 (1991).
8. R. Heumann., D. Lindholm, C. Bandtlow, *et al., Proc. Nat. Acad. Sci. USA*, **84**, 8735 (1987).
9. D. Lindholm, R. Heumann., M. Meyer, *et al., Nature*, **330**, 658 (1987).
10. D. Lindholm, R. Heumann., B. Hengerer, et al., J. Biol. Chem., **263**, 16348 (1988).
11. P. T. Manning, J. H. Russel, B. Simmons, *et al., Brain Res.*, **340**, No 1, 61 (1985).
12. R. Phillips and A. R. Rabson, *J. Clin. Lab. Immunol.*, **11**, 101 (1983).
13. T. Ravikumar, G. Steele, M. Rodrick, *et al.*, in: *Thymic Hormones and Lymphokines: Basic Chemical and Clinical Applications*, Washington (1988), p. 469.
14. L. M. Shabad, *J. Nat. Cancer Inst.*, **28**, № 6, 1305 (1962).
15. J. C. de la Torre and J. W. Surgeon, *Histochemistry*, **49**, № 2, 81 (1976).

EXPERIMENTAL BIOLOGY

Circadian Changes of the Hemocoagulation Indexes in Healthy Persons

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There have been few investigations of the circadian rhythm of hemocoagulation [2-5,8-11], and some of these studies were performed using obsolete techniques.

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In recent years refined methods of assaying hemocoagulation have been created, one of which is the anticoagulation test [13] in a modification [6] which assesses the kinetics of both the coagulation and anticoagulation processes. The hemolysate aggregation test [1] is suitable for studying platelet aggregation capacity.

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