# ROLE OF THE HEPATIC MACROPHAGAL SYSTEM IN LOWERING BLOOD LEVELS OF IMMUNE COMPLEXES DURING ADAPTATTON TO PERIODIC HYPOXIA

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The problem of immunocomplex pathology is currently assuming ever increasing importance in clinical practice, as is shown by the isolation of an extensive group of pathological states designated "immune complex diseases" [11].

It is now known that immune complexes (IC) play an important role in the pathogenesis of diseases such as atherosclerosis [6], multiple sclerosis and allergic encephalomyelitis [2], pollinoses [14], infectious-allergic and atopic bronchial asthma [15], and the allergic dermatoses [1]. In connection with the above facts we have recently shown that adaptation to periodic hypoxia in a hypobaric pressure chamber leads to marked reduction of the raised blood level of IC in patients with the atopic form of bronchial asthma, allergic dermatoses, and Hashimoto's autoimmune thyroiditis, and that this shift is accompanied by a marked therapeutic effect in these diseases [9, 10, 17]. However, the mechanism of the fall of the IC level in these clinical-physiological investigations was not fully explained. An important role in the removal of IC from the blood stream is played by the macrophagal system (MPS), and in particular, by the Kupffer cells of the liver [13], which can be activated by moderate hypoxia [5]. Accordingly we postulated that during adaptation to periodic hypoxia activation of MPS takes place, and that this plays a role in the lowering of the blood IC level in allergic and other diseases. The aim of the present investigation was to assess the effect of adaptation to periodic hypoxia on nonreceptor- and receptor-induced phagocytosis and on the digestive function of the mononuclear phagocytes in the liver.

## EXPERIMENTAL METHOD

Experiments were carried out on 120 male Wistar rats weighing 200-220 g. The animals were adapted to hypoxia by "ascent" to an "altitude" of 5000 m in a pressure chamber for 6 h daily for 40 days. Activity of cells of the hepatic MPS was estimated from the rate of clearance of the blood from intravenously injected ink [12], for this population of mononuclear phagocytes is known to be capable of removing 90% of the ink from the blood stream [13, 12]. The animal was anesthetized by intraperitoneal injection of urethane in a dose of 1 g/kg [3]. To 3 ml of a 3% solution of gelatin 2 ml of ink was added and the resulting mixture was injected in a dose of 0.26 ml/100 g body weight into a femoral vein. Blood for investigation was taken from the jugular vein in a volume of 0.1 ml 15 sec after injection of the ink, and thereafter every 3 min, into test tubes containing 3 ml of physiological saline with heparin (5 U/ml). The presence of ink in the samples was determined after centrifugation at 1000g for 15 min, spectrophotometrically at a wavelength of 650 nm. The result was expressed as a percentage of the initial quantity, the concentration of ink in the blood 15 sec after injection being taken as 100%.

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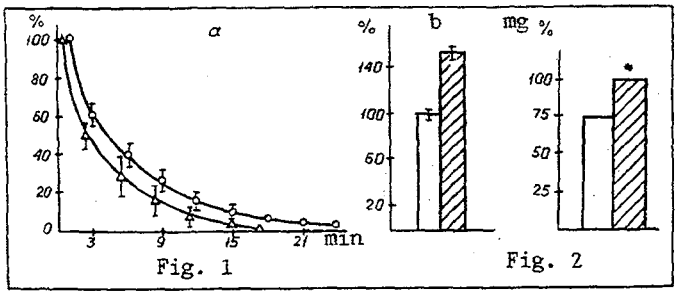

Fig. 1 Fig. 2

Fig. 1. Effect of adaptation to hypoxia on phagocytic activity of rat liver MPS: a) clearance of ink: triangles – animals adapted to periodic action of hypoxia; circles – intact animals (control); b) phagocytosis of aggregated IgG-FITC: shaded column – animals adapted to periodic hypoxia; unshaded – control.

Fig. 2. Effect of adaptation to periodic action of hypoxia on blood level of $C_3$-component of complement in children with bronchial asthma.

Receptor-induced phagocytosis of mononuclear phagocytes was assessed as ingestion of an analog of circulating IC, namely aggregated IgG, labeled with fluorescein isothiocyanate (IgG-FITC). For this purpose, a preparation of dry luminescent gamma-globulin against rabbit immunoglobulins (from the Gamaleya Institute of Epidemiology and Microbiology) was dissolved in 3 ml of physiological saline and aggregated for 20 min at 63°C [4]. The resulting solution was injected into the femoral vein of the previously anesthetized animals in a dose of 0.28 ml/100 g body weight. The animals were killed after 30 min by decapitation and the liver was perfused with physiological saline until it was yellow in color. A sample of tissue weighing 200-300 mg was homogenized in 5 ml of a 0.1% solution of Triton X-100 ("Merck") and centrifuged for 15 min at 9000g, after which 0.1 ml of supernatant was transferred into a test tube containing 3 ml of 0.1% solution of Triton X-100, and the fluorescence of the samples was measured on an MPF-4 spectrofluorometer (Hitachi), with wavelength of excitation of 495 nm and of emission of 522 nm. The results were expressed in units of fluorescence per gram of liver tissue.

To study the digestive function of the hepatic mononuclear phagocytes the animals were decapitated 2 and 6 days after intravenous injection of the aggregated IgG-FITC. Treatment of the material and measurement were carried out in the same way as for estimation of receptor-induced phagocytosis, fluorescence determined in the liver tissue homogenate 30 min after injection of aggregated IgG being taken as 100%.

The results were subjected to statistical analysis by Student's t test.

## EXPERIMENTAL RESULTS

The study of nonreceptor-induced phagocytosis, namely the ability of the Kupffer cells to ingest from the blood ink injected intravenously, showed that by the 9th minute more than 70% of injected ink had been ingested by the unadapted animals, and by the 24th minute virtually no ink could be detected in the blood. Adaptation of the animals to the periodic action of hypoxia led to an increase in the ingestive function of the phagocytic cells compared with unadapted animals (Fig. 1a). This increase was expressed as more rapid clearance of ink throughout the period of the investigation until differences between them were significant ($p < 0.05$). Since the rate of redistribution of ink between the blood and organs is an exponential function of time, it can be characterized by a value reflecting the half-clearance time of ink from the blood (HCT). In the adapted rats this parameter was 4 min, but only 2.5 min in the adapted rats.

Investigation of receptor phagocytosis, using aggregated IgG-FITC as analog of circulating IC showed that the intensity of fluorescence per unit mass of the liver 30 min after intravenous injection of labeled IgG in adapted animals was 1.6 times higher than in unadapted (Fig. 1b).

The results are thus conclusive evidence of the stimulating effect of adaptation to hypoxia on both nonreceptor and receptor phagocytosis of MPS in the liver. This important fact is in some agreement with existing data on the ability of adaptation to hypoxia to induce activation of immunologic processes [8], and it may probably be based on the character of restructuring of metabolism in the adapted animals, and linked primarily with intensification of protein biosynthesis [9]. This intensification may lead both to an increase in the blood concentration of nonspecific proteins ($\alpha_2$SB), enhancing the phagocytic activity of the stellate reticuloendotheliocytes of the liver and of the system of mononuclear phagocytes as a whole, and also to the more rapid restoration of cell membrane components utilized in the course of phagocytosis [13]. Whatever the concrete pathways of realization of this activating effect of adaptation on the macrophagal system of the liver, the fact that such activity does indeed take place may represent an important mechanism increasing the clearance of circulating IC and, consequently, lowering their blood level.

It was later discovered that reducing the concentration of circulating IC depends not only on the phagocytic function, but also on the digestive function of the hepatic MPS. The study of this function in our experiments showed that metabolism of aggregated IgG-FITC, phagocytosed by Kupffer cells, takes place as an exponential function. For instance, 24 h after its intravenous injection the residual content of the preparation in hepatic mononuclear phagocytes was 62.4 ± 1.7% of the injected dose: after 48 h it was 45.9 ± 1.7% and after 6 days 36.7 ± 1.1%. Preliminary adaptation to periodic hypoxia induced the digestive function of the hepatic mononuclear phagocytes, and this was reflected in the more rapid degradation and elimination of aggregated IgG-FITC from the Kupffer cells. In adapted animals, for instance, 24 h after intravenous injection of IgG-FITC its residual content was 53.7 ± 1.5%, falling after 6 days to only 27.0 ± 0.5%. It can be concluded from the results that adaptive induction of the hepatic macrophagal system is realized not only as activation of its ingestive capacity, but also of the catabolic activity of the mononuclear phagocytes toward aggregated immunoglobulins.

When the data on the role of activation of the phagocytic mechanism in lowering the level of circulating IC are evaluated, it has to be noted that the concept of "receptor phagocytosis" in relation to immune complexes is regarded from the standpoint not only of involvement of receptors of phagocytes for the Fc fragment of the immunoglobulins, but also, and predominantly, of their receptors for the C3b subcomponent of the complement system [7]. It is on account of C3b that the circulating IC can bind most effectively with phagocytes and be ingested by them. It must, however, be pointed out that the efficiency of binding of C3b to IgG by antibodies in the composition of IC is relatively low, and for that reason this interaction takes place only in the presence of a high blood level of C3b. In this connection, in a separate study using the method of radial immunodiffusion [16], with a test kit from the Institute of Sera and Vaccines (Czechoslovakia), blood levels of C3 were analyzed in children with bronchial asthma, before and after adaptation. The results showed that after the formation of adaptation the level of the C3-component of complement was significantly higher than initially (Fig. 2).

Thus, together with other factors, the increase in the C3b concentration in the blood may evidently play an important role in the activation of IC ingestion by MPS of the liver. This phenomenon as a whole evidently plays an important role in the mechanism of the therapeutic action of adaptation to periodic hypoxia in allergic diseases, and it deserves further study.

## REFERENCES


1. A. P. Belozorov, N. A. Goncharov, and G. S. Tserandis, Dermatol. Venerol. (Kiev), No. 17 (1988).
2. G. A. Vilkov, V. V. Martirosyan, L. I. Mezhova, and V. M. Mzokova, Zh. Nevropatol. Psikhiat., No. 2, 139 (1979).
3. V. V. Gatsura and A. S. Saratikov, Pharmacologic Agents in Experimental Medicine and Biology [in Russian], Tomsk (1977), p. 156.
4. B. Howard and T. Dacler, Lymphocytes: Isolation, Fractionation, Characterization, ed. by J. Katvik et al. [Russian translation], Moscow (1900), pp. 134-144.
5. A. S. Kaplanskii, "Immunologic and cytochemical study of defensive systems of the body in hypoxia and hyperoxia," Author's Abstract of Dissertation for the Degree of Doctor of Medical Sciences, Moscow (1974).
6. A. N. Klimov, A. P. Denisenko, and V. Ya. Nagornev, Immunoreactivity and Atherosclerosis, ed. by A. N. Klimov [in Russian], Leningrad (1986), pp. 107-128.

7. A. Ya. Kul'berg, Regulation of the Immune Response [in Russian], Irkutsk (1986), p. 224.
8. F. Z. Meerson, G. T. Sukhikh, and B. A. Frolov, Immunologiya, No. 3, 34 (1981).
9. F. Z. Meerson, V. P. Tverdokhlib, V. M. Boev, and B. A. Frolov, Adaptation to Periodic Hypoxia from Therapeutic and Preventive Aspects, ed. by O. G. Gazenko [in Russian], Moscow (1989), p. 70.
10. F. Z. Meerson, B. A. Frolov, et al., Patol. Fiziol., No. 3, 16 (1990).
11. R. V. Petrov, Immunology [in Russian], Moscow (1982), pp. 317-336.
12. I. Ya. Uchitel', Macrophages and Immunity [in Russian], Moscow (1978), pp. 180-181.
13. I. S. Freidlin, The Mononuclear Phagocyte System [in Russian], Moscow (1984), p. 272.
14. R. Heigl, V. Wekn, P. Koldovski, et al., Allergologie, **9**, No. 9, 402 (1986).
15. M. Kraus-Filarska, J. Malolopszy, R. Jakonska, and W. Kopec, Pol. Tyg. Lek., **40**, No. 12, 353 (1985).
16. C. Mancini, A. O. Karvonara, and J. F. Heremans, Immunochemistry, **2**, No. 2, 235 (1965).
17. F. Z. Meerson, CV World Rep., **3**, 116 (1990).