Physiol. Res. 42: 201 – 205, 1993

Perinatal Hypoxia Suppresses Immune Response of Adult Rats

M. VÍZEK, M. DOSTÁL[1], D. SOUKUPOVÁ[1]

Institute of Pathophysiology, Second Medical Faculty, Charles University, and [1]Institute of Experimental Medicine, Academy of Sciences of Czech Republic, Prague

Received March 12, 1993
Accepted April 21, 1993

Summary

We tested the effect of perinatal (one week prenatal and one week postnatal) normobaric hypoxia on the immune response of rats in their 9th week of life. We found that perinatally hypoxic rats produced less serum antibodies after sequential immunization with ovalbumin and sheep red blood cells. Also phagocytosis of HEMA microparticles by neutrophil leukocytes from perinatally hypoxic rats was depressed as well as the oxidative burst of their peritoneal macrophages and neutrophils. These results demonstrate that perinatal hypoxia has an important effect on the immune system of the rat.

Key words

Normobaric hypoxia – Development – Rat – Immune response

Introduction

The evidence that exposure to high altitude impairs immune competence of humans as well as animals is growing (see Meehan *et al.* 1987, 1988). Crucial role between mechanisms causing this blunting of immune response is presumably played by hypoxia. We hypothesized that hypoxia applied in periods of increased sensitivity of the immune system to noxious stimuli may have important delayed effects. Therefore we designed a preliminary study testing effects of perinatally applied normobaric hypoxia on immune response of adult rats.

Material and Methods

Experimental animals and protocol: Four pregnant Wistar rats were placed into normobaric hypoxic chamber (F_IO_2 = 0.12, Leach *et al.* 1977) one week before expected delivery.

The actual time spent in the chamber before delivery ranged from 6 to 8 days. Newborn rats were kept with their mothers in the same hypoxic environment for another week. For the next 7 weeks the experimental animals lived in room air. Twelve males were randomly chosen for experiments. Twelve controls were born and kept in the room air. All animals were weaned at the age of 4 weeks.

At the age of 8 weeks six experimental and six control rats were sequentially immunized by bovine serum albumin (BSA, day 0, 100 micrograms, emulsified in an equal volume of Freund's complete adjuvans injected at the base of the tail) and sheep red blood cells (SRBC, dose 2.10^9, injected i.p. on day 3). Other six experimental and six control rats were immunized by ovalbumin (OVA, day 0, 100 micrograms) and SRBC (day 5) injected in the same way and doses as in the combination BSA+SRBC. The immune responses to BSA+SRBC and OVA+SRBC were assayed on day 8 and 10, respectively.

The serum hemagglutinating antibodies against SRBC were determined by microtitration. The spleen anti-SRBC class IgM antibodies were determined using lymphocyte mediated SRBC hemolysis (Simpson and Gozo 1978). Serum titres of IgG and IgM antibodies to ovalbumin were determined using the enzyme-linked immunosorbent assay (Vos *et al.* 1970). The respiratory burst of peritoneal macrophages was assayed after elicitation with

thioglycolate and *in vitro* triggering with opsonized zymosane using reduction of iodonitrotetrazolium (INT test). Phagocytic activity of peripheral blood neutrophils was quantitated using HEMA particles (Větvička *et al.* 1982). The Mann-Whitney U test was used for statistical evaluation of the data.

Results

Body weights and numbers of red and white blood cells in control and experimental rats did not differ.

Antibody production was in perinatally hypoxic rats depressed in the case of immunization by OVA and SRBC. Serum titres of the total immunoglobulins and of the class IgM antibodies (Table 1) show that control animals have produced at least twice as much anti-SRBC antibodies than perinatally hypoxic rats. Also the data about the spleen weight, numbers of spleen nucleated cells and IgM production by the spleen cells suggest an impairment of humoral response to SRBC. The response to OVA did not differ. Antibody production in response to immunization by BSA and SRBC was in both groups similar.

The oxidative burst (INT test) of peritoneal macrophages and neutrophils was depressed in the experimental rats after both types of immunization (Fig. 1). Phagocytosis of HEMA microparticles was assayed only in rats immunized by OVA+SRBC. 30.2 % of neutrophil leucocytes were found to phagocytize the particles in the control group, and only 19.4 % in the experimental group ($P<0.05$).

Table 1
Humoral immune response after sequential immunization by OVA and SRBC

	No.	SERUM					SPLEEN		
			anti-SRBC		anti-OVA		weight	cells	anti-SRBC
		Total	IgG	IgM	IgM	IgG			IgM
Mean		titres[a]			(abs.)[b]		(mg)	(10^6)	(abs.)[c]
Control	6	5.5	2.4	3.0	0.35	1.39	1025.5	541.9	1.26
Hypoxia	6	4.1[1]	2.9	1.3[2]	0.48	1.36	707.3	83.8[1]	1.04

[a] : $-\log_2$ of serial dilution of the serum; [b] : absorbance at the 1:64 dilution of the serum; [c] : dilution of spleen cells 1:400; [1] :$P< 0.01$, [2] : < 0.001 (Mann-Whitney U test)

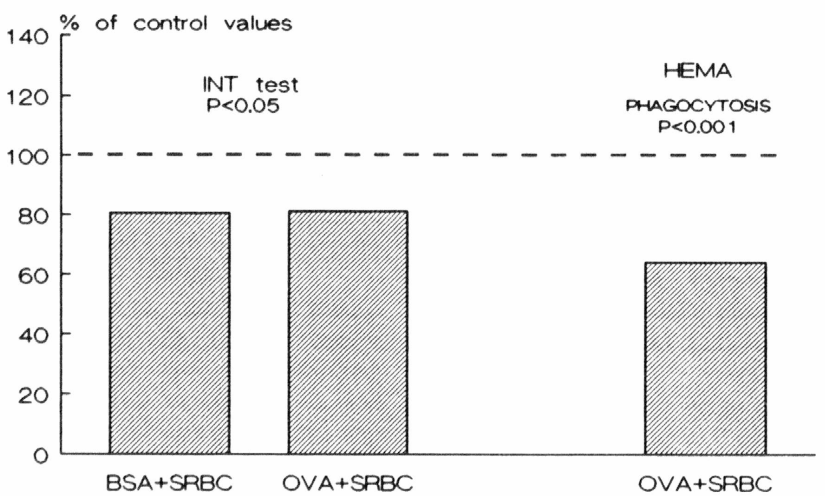

Fig. 1
Oxidative burst of peritoneal macrophages and neutrophils (INT test) and phagocytosis of HEMA microspheres by peripheral blood neutrophils.

Discussion

The present study shows that the rats born in hypoxia and then raised in normal air developed the impairment of immune response. As far as we are aware of, our study is the first one to suggest that perinatal hypoxia compromises the immune response of adults.

The tests we have used are those commonly used in immunotoxicology and were aimed at assaying simultaneously nonspecific as well as specific responses.

Serum titres of antigen specific immunoglobulins providing the information about the ability to generate a primary immune response are a sensitive indicator of immune dysfunction (National Research Council 1992). The fact that antibody production was in perinatally hypoxic rats depressed against SRBC and not against OVA (i.e. against the second of two consecutive stimuli only) suggests that a more intensive challenge is required to uncover a depressed reserve capacity of the immune system. The different time delay between the first and second challenge could probably explain the difference in the response of perinatally hypoxic rats to combinations BSA + SRBC and OVA + SRBC.

The *in vitro* ingestion of innert HEMA particles by peripheral blood neutrophils informs about the adhesion and ingestion phases of phagocytosis. The decreased phagocytosis of these particles by neutrophils derived from perinatally hypoxic rats indicates an impairment of their function. Ingestion of foreign materials is followed by a burst of metabolic activity. Decreased reduction of INT by activated macrophages and neutrophils from experimental animals reflects their lower metabolic activity induced by stimulation (Neveu 1986).

Considerable evidence that high altitude affects the immune response has been recently reviewed by Meehan *et al.* (1987). Noxious effect of hypoxia is the most frequently offered explanation of this finding, but the mechanism of modulation of the immune system by hypoxia remains unclear. Hypoxia could act as a stressor. Different types of stress were reported to affect immunity (Stein *et al.* 1985). Perinatal hypoxia could also change hormonal regulation and many hormones have been reported to modulate immune function (Stein *et al.* 1981). Since there is a good evidence that also neural mechanisms are involved in regulation of immunity (Cross *et al.* 1982), hypoxia-induced changes in CNS could affect immunity. Interesting possibility is a depression of immune response caused by immature erythroid cells (Er suppressors) humorally inhibiting B cell proliferation (Tsyrlova 1991).

It was well established that a developing immune system is rather sensitive to distressing factors (reviewed by Roberts and Chapman 1981, Schmidt 1984). Therefore, direct effects of hypoxia on the offspring should be considered first. Nevertheless, alteration of the development of immune system due to modulation of maternal immunity has been reported (Binder *et al.* 1985) and thus maternally-mediated effects of hypoxia are also possible.

The fact that we cannot provide an explanation of the mechanism of blunting the immune response after prenatal and perinatal exposure to hypoxia stresses the importance of further studies.

References

BINDER P., GENDRE F., HUART B., PAUCOD J.-CH., DESCHAUX P., FONTANGES J.: Immunomodulation in offspring of mice after neonatal immunostimulation of mothers or newborn mice. *Tohoku J. Exp. Med.* **146**: 379–383, 1985.

CROSS R.J., BROOKS W.H., ROSZMAN T.L., MARKESBERY R.: Hypothalamic-immune interactions. Effect of hypophysectomy on neuro-immunomodulation. *J. Neurol. Sci.* **53**: 557–563, 1982.

LEACH E., HOWARD P., BARER G.R.: Resolution of hypoxic changes in the heart pulmonary arterioles of rats during intermittent correction of hypoxia. *Clin. Sci. Mol. Med.* **52**: 153–162, 1977.

MEEHAN R.T., DUNCAN U., NEALE L.: Immune suppression at high altitude. *Ann. Emerg. Med.* **16**: 974–979, 1987.

MEEHAN R.T., DUNCAN U., NEALE L., TAYLOR G., MUCHMORE H., SCOTT N., RAMSEY K., GOLDBLUM R., HOUSTON CH. .: Operation Everest II: Alterations in the immune system at high altitudes. *J. Clin. Immunol.* **8**: 397–406, 1988.

NATIONAL RESEARCH COUNCIL: Biologic Markers in Immunotoxicology, National Academy Press, Washington, D.C. 1992.

NEVEU P.J.: The mononuclear phagocyte system. *Bull. Inst. Pasteur* **84**: 23–66, 1986.

ROBERTS W., CHAPMAN J.R.: Concepts essential to the assessment of toxicity to the developing immune system. In: *Developmental Toxicology*, C.A. KIMMEL and J. BUELKE-SAM (eds), Raven Press, New York, 1981, pp. 167–190.

SCHMIDT R.R.: Altered development of immunocompetence following prenatal insult: A timely review. *J. Am. Coll. Toxicol.* **3**: 57–72. 1984.

SIMPSON M.A., GOZO J.J.: Spectrophotometric determination of lymphocyte mediated sheep red blood cell hemolysis in vitro. *J. Immunol. Methods* 21: 159–165, 1978.

STEIN M., KELLER S., SCHLEIFER S.: The hypothalamus and the immune response. In: *Brain, Behavior and Bodily Disease*. H. WEINER, M.A. HOFER, and A.J. STUNKARD (eds), Raven Press. New York. 1981.

STEIN M., KELLER S., SCHLEIFER S.: Stress and immunomodulation. The role of depression and neuroendocrine function. *J. Immunol.* **135**: 827–883, 1985.

TSYRLOVA I.G.: Erythroid immunosuppressor cell (Er suppressor)and their role in the regulation of immunity. *Vest. Akad. Med. Nauk SSSR* **12**: 34–9, 1991.

VĚTVIČKA, V., FORNŮSEK, L., KOPEČEK, J., KAMÍNKOVÁ, J., KAŠPÁREK L., VRÁNOVÁ M.: Phagocytosis of human blood leukocytes: a simple micromethod. *Immunol. Lett.* **5**: 97–99, 1982.

VOS J.G., BOERKAMP., BUYS J., STEERENBERG P.A.: Ovalbumin immunity in the rat: Simultaneous testing of IgG, IgM and IgE response measured by ELISA and delayed-type hypersensitivy. *Scand. J. Immunol.* **12**: 289–295, 1970.

Reprint Requests
Dr. M. Vízek, Institute of Pathophysiology, 2nd Medical Faculty, Charles University, Plzeňská 221, 150 00 Prague 5, Czech Republic.