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THE INFLUENCE OF ENDOGENOUS HYPOCORTICISM ON THE REPLICATION OF INFLUENZA VIRUS IN THE MOUSE LUNGS AND THE PRODUCTION OF SPECIFIC ANTIBODIES

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Summary. — The replication of pathogenic influenza virus A/PR/8/34 in the lungs and the synthesis of virus-neutralizing (VN) and haemagglutination-inhibition (HI) antibodies has been studied in mice with endogenous hypocorticism induced by a bilateral adrenalectomy. The adrenalectomized mice appeared to be more susceptible to influenza infection as compared to the mock-operated ones. This was evident from earlier deaths and higher death rate in mice inoculated with 50 EID_{50}, 1000 EID_{50}, and 6000 EID_{50} of the virus, respectively. A tendency towards decrease of specific antibody titres and the resistance to reinfection with influenza virus A/PR/8/34 was also observed.

Key words: *adrenalectomy*; *endogenous glucocorticoids influenza*; *virus A/PR/8/34*; *virus-neutralizing and haemagglutination-inhibition antibodies*

Introduction

Glucocorticoids are known to exert an anti-inflammatory effect. As potent immunosuppressors, however, they can inhibit the immunological responses and thereby activate viral and bacterial infections. There is much controversy in literature on the effect of glucocorticoids on virus replication. In the experiments of Kalter *et al.* (1951) therapeutic doses of ACTH and cortisone suppressed the replication of influenza virus in the mouse lungs. Hydrocortisone enhanced the expression of HTLV-III in human lymphocyte culture and the synthesis of type C endogenous retroviruses in mouse fibroblast culture (Markham *et al.*, 1986; Paran *et al.*, 1973). The injection of cortisone to mice after the inoculation with type II poliovirus decreased the inflammation but increased the virus titres in the animal brain (Nosik, 1959). Most papers studied the influence of exogenous glucocorticoids on the virus, although a natural infection may be regulated by endogenous glucocorticoids. The latter are produced by the adrenal cortex and their level varies in the course of the infectious process. The purpose of the present paper

has been to study the replication of influenza virus in albino mice with endogenous hypocorticism and the synthesis of specific antibodies and resistance to reinfection with the pathogenic influenza virus A/PR/8/34.

Materials and Methods

Animals. The experiments were carried out in male hybrids (CBA × C57BL/6) aged 3 or 4 months. The mice were obtained from inbred animals nursery of the Academy of Medical Sciences of U.S.S.R. (Stolbovaya). Altogether 170 mice were used in 2 trials.

Induction of endogenous hypocorticism. To produce endogenous hypocorticism bilateral adrenalectomy was performed since the previous data indicated (Semenkov and Molotkov, 1974; Semenkov and Afinogenova, 1982) that the corticosterone level was half as high in adrenalectomized animals as in the intact ones. Adrenal glands were removed through a median skin incision to lumbar area. The right and left subcostal muscles were dissected and the upper kidney poles were then approached in order to extract the adrenals. Sham surgery was simulated in control animals. This included the same manipulations except for the extirpation of the adrenals. The mice operated on were given 0.85% saline.

Viruses and infection. Influenza virus strains A/PR/8/34 (H1N1) and A/Krasnodar/101/59 (H2N2) were grown in chick embryos. The infectivity of influenza viruses was determined by infection of 9 or 10-day-old chick embryos into the allantoic cavity. The virus content was determined by haemagglutination activity after incubation of the embryos at 37 °C for 48 hr. The infectivity was expressed in embryo infectious units (EID_{50}). In the 1st series experimental and control animals were infected intranasally on day 3 after surgery with the strain A/PR/8/34 in doses 5 EID_{50}, 50 EID_{50}, and 6000 EID_{50}. In the 2nd series the mice were infected intranasally on day 3 after the surgery with either live or killed influenza virus A/PR/8/34 in a dose of 1000 EID_{50}. The virus was inactivated by heating in the water bath at 56 °C for 30 min. In this series some adrenalectomized mice were infected with live virus A/Krasnodar/59. For the assessment of the developed resistance to the challenge with the pathogenic virus, the mice were reinfected intranasally with A/PR/8/34 virus in a dose of 6000 EID_{50} 30 days after the first influenza virus administration both in the 1st and 2nd experimental series. The concentration of the virus in the lungs was determined on day 3 after the first and second infection by titration of the lung tissue suspension in 10-day-old chick embryos with a correction for the weight of the lungs. The virus titres were mean values of the titres in the lung tissue of 2 or 4 mice.

Serologic tests. The serum from experimental and control mice were collected on day 19 to 25 after the first infection with influenza virus. The sera were inactivated by heating at 56 °C for 30 min and titrated in a HI and VN tests according to WHO protocols (1959). The titres were averaged over titrations in 2 or 4 mice.

Proliferative response of spleen lymphocytes. Seventy-two hr after intranasal infection with the virus A/PR/8/34 (1000 EID_{50}) spleen cells from mice were collected. The lymphocytes were isolated from the spleen cell suspension by Ficoll-Pack density gradient centrifugations (Q = 1.077) at 1500 rev/min for 40 min (contrifuge LU 418H, Hungary). The lymphocytes were rinsed in Hanks' solution and cultured in medium RPMI-1640 in a concentration of 10^6 cells/ml in the presence of 10% foetal calf serum, HEPES, 2-mercaptoethanol, L-glutamine, and gentamycine in 5% CO_2 at 37 °C. The lymphocytes were stimulated for 72 hr by inactivated purified influenza virus. The proliferative response of lymphocytes was measured by incorporation of ^{3}H-thymidine in 3 parallel cultures (for details see Lavrov *et al.*, 1987).

Results

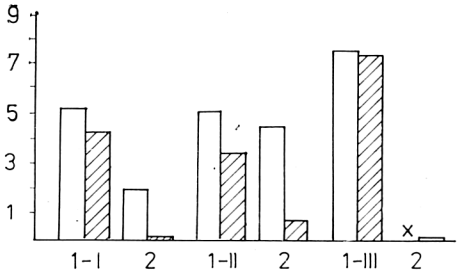
First trials

It can be seen in Fig. 1 that mean titres of influenza virus A/PR/8/34 were higher in the lungs of adrenalectomized mice than in control ones with either 5 EID_{50} or 50 EID_{50}. After infection with a dose of 6000 EID_{50} the

Fig. 1.

Replication of influenza A virus in the lungs of mice with endogenous hypocorticism

Abscissa: white columns — virus titres in the lungs of adrenalectomized mice. Shaded columns — virus titres in the lungs of mock-operated mice. 1 — primary injection of the virus in doses: I — 5 EID_{50} per mouse, II — 50 EID_{50} per mouse, III — 6000 EID_{50} per mouse; 2 — virus challenge with a dose of 6000 EID_{50} per mouse. Cross indicates that the virus titres were not determined because these mice died after primoinfection. Ordinate — virus titres (log EID_{50}).



titres were essentially equal. Infection with 5 EID_{50} of the A/PR/8/34 strain caused deaths neither of experimental nor of control animals. The dose of 50 EID_{50} led to the death of 3 adrenalectomized mice out of 12, whereas all control mice survived. The dose of 6000 EID_{50} killed all adrenalectomized mice by day 9, the first deaths occurring on day 5. In the mock-operated animals the first deaths occurred 2 days later, and 4 mice out of 12 survived. Upon reinfection of mice after 30 days with 6000 EID_{50} of strain A/PR/8/34 a marked suppression of virus replication was observed in the lungs of both adrenalectomized mice and of the mock-operated ones. Less virus multiplication was found, however, in mock-operated mice. Mean titres of virus-

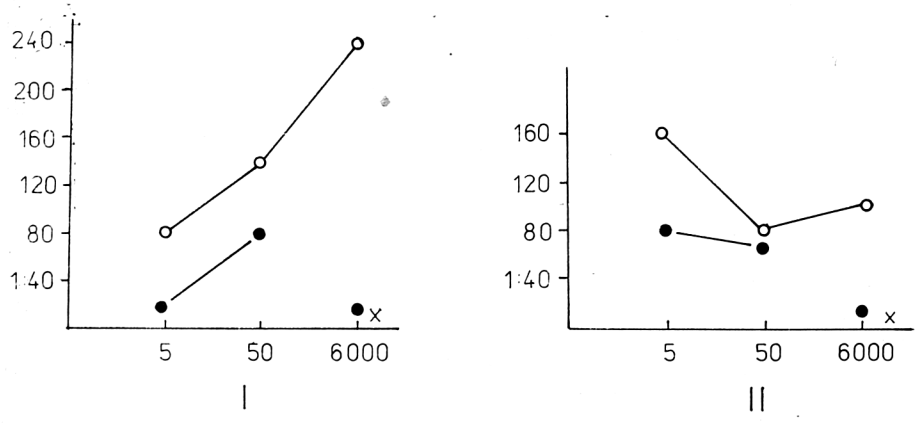


Fig. 2.

The titres of virus-neutralizing (I) and haemagglutination-inhibition antibodies (II) in mice with endogenous hypocorticism

Abscissa: virus doses for primary infection (EID_{50}/mouse), ordinate — antibody titres. Black circles — adrenalectomized mice, white circles — mock-operated mice. The cross indicates that antibody titres were not determined in the adrenalectomized mice because they died after primoinfection. Ordinate: serum dilution reciprocals.

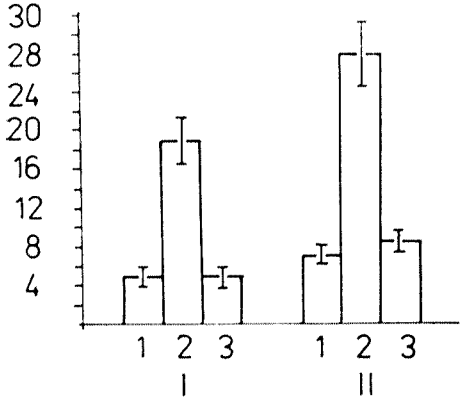


Fig. 3.

Proliferative response of spleen lymphocytes in mock-operated mice (I) and in adrenalectomized mice (II) with endogenous hypocorticism

Abscissa: 1 — spontaneous proliferation, 2 — proliferation in response to the stimulation of lymphocytes with strain A/Krasnodar/101/59, 3 — in response to the stimulation with strain A/PR/8/34. Ordinate — incorporation of ^{3}H-thymidine ($\times 10^3$ counts/min).

neutralizing antibodies to strain A/PR/8/34 were higher in the mock-operated animals than in the adrenalectomized mice. The HI antibody titres were also higher in the former after a dose of 5 EID_{50}, shereas after injecion of 50 EID_{50} they were essentially identical (Fig. 2).

Second trial

Because in the first series there was no difference between virus titres in the lungs of adrenalectomized and control animals inoculated with high doses of A/PR/8/34 (6000 EID_{50}), 1000 EID_{50} of A/PR/8/34 (less than the sublethal dose) was used for intranasal infection in the 2nd series (Table 1). After administration of this dose no marked difference between the virus titres in the lungs of experimental and control animals was observed and the death rates were equal (40%), but first deaths occurred earlier in the adrenal ectomized animals. Similarly to the first series, the mean titres of virus-neutralizing antibodies tended to be higher in the mock-operated mice than in the adrenalectomized ones, whereas the HI antibody titres did not show any difference. Influenza virus replication in the lungs was totally suppressed after reinfection with 6000 EID_{50} of the strain A/PR/8/34 both in the mice that underwent adrenalectomy as well as in the control animals. It is noteworthy that no specific antibodies were detected in the serum in response to the administration of live virus A/Krasnodar/101/59 or the killed A/PR/8/34 virus, either in adrenalectomized or in control mice. After reinfection with 6000 EID_{50} of A/PR/8/34 the replication of the virus was not suppressed (Table 1, groups 3 and 4). After stimulation with the specific strain A/PR/8/34 the proliferative response of spleen lymphocytes of mice with endogenous hypocorticism appeared to be 1.7 times higher than in controls; after injection of the heterologous strain A/Krasnodar/101/59 it was 1.5 times higher. The spontaneous proliferation of lymphocytes increased 1.3 times (Fig. 3).

Table 1. Replication of influenza virus strains A/PR/8/34 (H1N1) and A/Krasnodar/101/59 (H2N2) in the lungs of adrenalectomized mice and the levels of humoral antibodies

Group	No. of mice		Virus dose (EID_{50}) for primary infection with strains			Virus titres after infections		Specific antibodies	
	adrenal-ectomized	after simulated surgery	A/PR/8/34		A/Krasnodar// 101/59	first	second challenge	VN	HI
			live	killed	live				
1	12	—	1000	—	—	7.0; 6.0	0; 0	1/20	1/60
2	—	12	1000	—	—	8.0; 6.5	0; 0	1/40	1/60
3	12	—	—	1000	—	0; 0	7.2; 6.5	0	0
4	10	—	—	—	1000	4.0; 3.5	7.5; 7.2	0	0

Note. Specific antibody titres were determined only after primoinfection with influenza virus.

Discussion

It has been shown that the increased death rate in adrenalectomized mice with endogenous hypocorticism was in most cases associated with elevated virus titres in the lungs as compared with the control values. The increased susceptibility of mice with endogenous hypocorticism to influenza infection was most marked after infection with the doses of 5 EID_{50} and 50 EID_{50} of the strain A/PR/8/34, whereas upon inoculation of the dose 1000 EID_{50} the death rate of the experimental and control animals was similar. Yet, the first deaths occurred earlier in adrenalectomized mice. It has been previously shown (Semenkov and Molotkov, 1974; Semenkov, 1985) that endogenous hypocorticism enhances the immune responses of T-cells to strong and weak graft antigens. This enhancement of immunity seems to be not sufficient, however, for suppression of the virus replication. It should be taken into account that during endogenous hypocorticism the immunopathologic responses of T-cells can develop in parallel with the enhancement of the inflammatory process. It is possible that glucocorticoids can modify the replication of viruses by acting upon the function of the virus-susceptible cells.

High level of endogenous glucocorticoids can cause an enhanced virus replication owing to suppression of immune responses and inhibition of the synthesis of interleukine-1 and interleukine-2, and of the immune interferon mRNA (Arya *et al.*, 1984; Bochner *et al.*, 1987). A low level of endogenous glucocorticoids promotes the intensification of the production of interleukine-1 (the inflammation factor) and of interleukine-2 as an anabolic cell growth factor. This may create prerequisites for interaction of the virus with susceptible cells and for its replication.

Our results point at increased proliferative response of spleen lymphocytes to specific and nonspecific virus antigens in mice with endogenous hypocorticism. In adrenalectomized animals, however, no increase of virus-neutralizing or HI antibody titres has been observed at any infection dose of A/PR/8/34 virus. The synthesis of humoral antibodies to influenza virus is thymus-dependent (Callard, 1979). It cannot be ruled out that a certain level of endogenous glucocorticoids is needed for differentiation of cortisone-resistant subpopulations of T-lymphocytes acting in cooperation with B-lymphocytes. After primary infection with 5 EID_{50} or 50 EID_{50} of A/PR/8/34 the induction of resistance to reinfection with a lethal dose of this virus (6000 EID_{50}) was easier to achieve in the mice after simulated surgery than in adrenalectomized mice. After primary infection of mice with 1000 EID_{50} of A/PR/8/34 the induction of resistance to reinfection was identical in adrenalectomized and control mice.

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