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CYCLIC AMP IN MACROPHAGES, INTESTINAL MUCOSA, AND BLOOD PLASMA OF GERMFREE AND ORDINARY ANIMALS

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In recent years much attention has been paid to the system of cyclic nucleotides in the regulatory mechanisms of immunity of resistance to infection [2, 4, 6]. Germfree animals with their intact immune system constitute an adequate object for studying the role of the microbial factor in the development of the nucleotide-cyclase mechanisms of cellular reactivity of the host organism.

The object of this investigation was to study, in comparative experiments on germfree and ordinary animals, the role of natural microbial contamination of the host organism on ability to form cyclic AMP in the intestinal mucosa and macrophages.

EXPERIMENTAL METHOD

Germfree and ordinary C3H/He mice aged 3-4 months and outbred guinea pigs aged 2-3 weeks were used. The animals were kept in Trexler germfree isolators. During work with animals in germfree isolators, the rules established in [1] were observed. The germfree animals were kept, fed, and subjected to microbiological control in accordance with the general demands of gnotobiological technology [3].

The cyclic AMP content was determined in peritoneal macrophges, the intestinal mucosa, and the blood plasma of intact animals and also in the course of administration of Escherichia coli 055 lipopolysaccharide (LPS). The LPS was isolated by the water–phenol method [8] and purified on a Spinco ultracentrifuge at 105,000g.

The effect of LPS on the adenylate cyclase of the intestinal mucosa was studied by the method of local application in an isolated loop of small intestine of germfree guinea pigs. For this purpose, under ether anesthesia a loop of small intestine was isolated in the animals and 1000 μg of LPS in a volume of 0.5 ml of 0.14 M NaCl was injected into a segment of it (8 cm long), isolated by means of silk ligatures. Only physiological saline was injected in control experiments. The animals were killed after 30 and 60 min and scrapings of intestinal mucosa were obtained together with blood plasma. Weighed samples of the scrapings were homogenized in 1 ml 5% TCA and were extracted 5 times with 2 volumes of ether, after the addition of 0.1 ml 1 N HCl. Cyclic AMP was determined by the competitive binding method [5]. The values obtained for the cyclic AMP content in picomoles were expressed per 100 mg tissue and per milliliter of blood plasma.

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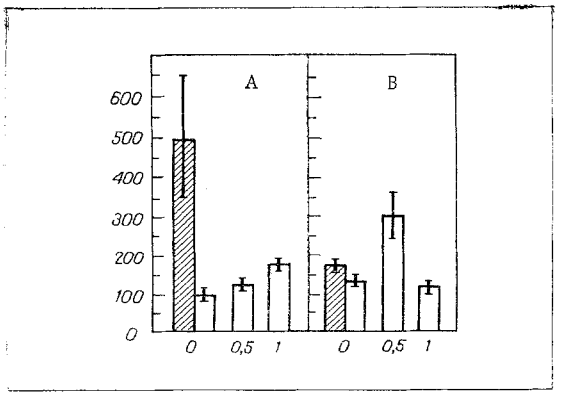

Fig. 1. Cyclic AMP content in intestinal mucosa and blood plasma of germfree and ordinary guinea pigs. Abscissa, time of injection of LPS from E. coli 055 (in h); ordinate, cyclic AMP concentration (in pmoles/100 mg tissue/ml plasma). A) Intestinal mucosa, B) blood plasma. Shaded columns represent ordinary animals; unshaded columns germfree animals at different times after injection of LPS.

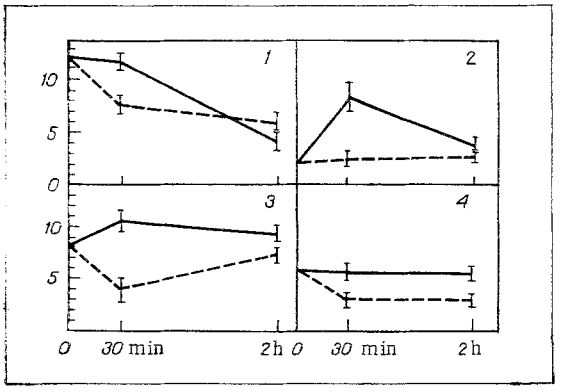

Fig. 2. Effect of LPS from E. coli 055 on cyclic AMP formation in macrophages of germfree and ordinary mice. 1) Extracellular medium of germfree mice, 2) macrophges of germfree mice, 3) extracellular medium of ordinary mice, 4) macrophages of ordinary mice. Continuous line – cyclic AMP concentration after stimulation by LPS, broken line – control (spontaneous production of cyclic AMP without stimulation by LPS).

To determine the cyclic AMP level in the macrophages, unstimulated peritoneal cells obtained by irrigation of the peritoneal cavity of the mice with Hanks' solution were used. The cell suspension (over 95% of the cells were macrophages) was washed. When cells were obtained from the mouse peritoneal cavity, the number of macrophages obtained from ordinary animals was three times greater ($6 \cdot 10^6$/ml) than from germfree animals ($2 \cdot 10^6$/ml). To ensure identical experimental conditions the number of cells taken from the germfree and ordinary animals was equalized. LPS was added to the resulting suspension of washed macrophages at the rate of 50 μg per 10^6 cells. Cyclic AMP levels were determined 30 min and 2 h after the beginning of interaction between the macrophages and LPS. Samples measuring 0.5 ml were taken at the above-mentioned intervals from the cell suspension. Cells sedimented by centrifugation at 1000g for 5 min were homogenized in a glass homogenizer. Cyclic AMP was determined by the above method with conversions of the cyclic AMP concentration to picomoles/10^6 macrophages.

Altogether 10 germfree and 10 ordinary mice and also 15 germfree and 15 ordinary guinea pigs were used.

EXPERIMENTAL RESULTS

The cyclic AMP level found in the intestinal mucosa of the intact guinea pigs was 5 times higher than in germfree animals (Fig. 1). Under the influence of LPS an increase was observed in the cyclic AMP content of the intestinal mucosa of the germfree guinea pigs after 30 min and 1 h. The cyclic AMP level in the plasma of the ordinary animals also was higher than in that of the germfree animals. A sharp rise in the cyclic AMP level (more than twofold) was observed 30 min after injection of LPS, falling to its initial level after 1 h of observation.

In the macrophages of the germfree mice 30 min after injection of LPS a significant (fourfold) increase in the cyclic AMP content was observed. In ordinary animals the doses of LPS used induced the formation of practically no cyclic AMP. In macrophages of ordinary animals, synthesis of cyclic AMP was equal to its secretion into the surrounding medium. The peak of formation of cyclic AMP was observed after 30 min, after which the level of this cyclic nucleotide fell (Fig. 2).

The results show that LPS stimulates cyclic AMP synthesis, and more especially in germfree animals. It can be tentatively suggested that in the latter, in which the spectrum of biological stimuli, primarily of microbial origin, is more limited, adenylate cyclase is less well balanced, and this is reflected in increased formation of cyclic AMP. It must be remembered that the cyclic AMP levels are determined by combined activity of both adenylate cyclase and phosphodiesterase. The fact that cyclic AMP synthesis in the intestinal mucosa of the germfree animals did not reach the values found in ordinary, contaminated animals, indicates the stimulating role of other microorganisms and their biologically active products in the formation of this cyclic nucleotide. The ability of the microbial cells themselves to synthesize their own cyclic AMP must also be borne in mind, and this also would account for the increased levels of cyclic AMP in the intestinal mucosa of the ordinary animals compared with the germfree animals.

The reduced content of cyclic AMP in intact germfree animals and the inadequate response of the cyclic nucleotide system to stimulation by products of microbial origin can also explain certain special features of immunobiologic reactivity in germfree animals and, in particular, depression of their resistance to infection. Disturbances of intracellular levels of cyclic nucleotides are known to lead to a reduction of immunocompetence [7]. The increase in cyclic AMP formation under the influence of LPS must be regarded as a biologically purposive response protecting the animal against excessive response to extremal biotic factors of the microbial environment. Through the "blocking" of various cellular functions cyclic AMP protects the body against the dangerous consequences of an uncontrolled immune response [2], thereby defining the important role of cyclic nucleotides in the regulation of homeostasis of the body.

The results show that cyclic AMP formation in the intestinal mucosa and peritoneal macrophages is dependent on microbial stimulation. The rise in the cyclic AMP levels in the blood plasma of germfree animals after stimulation by LPS may reflect the participation of this cyclic nucleotide in nonspecific reactions of the host organism to microbial stimulation, which resembles shock in type (microbial or endotoxin shock). These findings are in accordance with modern views on the role of cyclic nucleotides in the regulation of immunity and of the homeostatic reactions of the body [2, 4, 6].

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