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EFFECT OF CONTAMINATION OF GERMFREE GUINEA PIGS BY INDIVIDUAL MEMBERS OF THE INTESTINAL MICROFLORA ON ANTIBODY AND COMPLEMENT LEVELS

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UDC 574.24:576.85.083.5:576.851.48.097.5

On the 3rd day after birth germfree guinea pigs were contaminated by one of the following representatives of the normal intestinal microflora: Bacillus mesentericus, Bacillus subtilis, Staphylococous albus, and Streptococcus faecalis. The levels of antibodies against the microorganisms used for monocontamination and also against Escherichia coli 055, which is pathogenic for guinea pigs, and the serum complement levels were studied in the animals at the age of 2 weeks. Contamination of the guinea pigs by B. mesentericus and B. subtilis did not significantly change the antibody levels against these microorganisms, whereas S. albus and S. faecalis appreciably stimulated antibody formation. Similar results were obtained with respect to E. coli 055. The complement level was significantly increased by the spore-bearing aerobes and by S. albus.

KEY WORDS: germfree animals; normal microflora; antibodies; complement.

Experiments on germfree animals have established that the normal microflora stimulates the immune system of the host [3,4,6]. An interesting aspect of these investigations was the study of the immunogenic properties of individual members of the normal microflora.

The object of this investigation was to study the effect of oral contamination by individual members of the intestinal microflora on antibody formation against hose microorganisms and against Escherichia coli 055, and also the serum complement level of the germfree guinea pigs.

EXPERIMENTAL METHOD

Germfree and monocontaminated animals (6-10 in each group) aged 2 weeks were used.

Germfree guinea pigs were obtained from the mothers toward the end of gestation by caesarian section by a sterile isolation technique [3]. The animals were reared on the BMS-1 sterile diet developed in the writers'

Research Laboratory of Experimental Biological Models, Academy of Medical Sciences of the USSR, Moscow Region. (Presented by Academician of the Academy of Medical Sciences of the USSR A. M. Chernukh.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 9, pp. 1100-1102, September, 1976. Original article submitted November 28, 1975.

TABLE 1. Antibodies Against Individual Members of Intestinal Microflora in Serum of Germfree and Monocontaminated Guinea Pigs ($\log_2$)

Microorganism (antigen)	Animals donating serum		P
	Germfree	Monocontaminated	
Bac. mesentericus	1,44±0,11	1,64±0,25	>0,05
Bac. subtilis	0.74±0,21	1,66±0,12	<0,01
S. albus	4,58±0,15	6,47±0,14	<0,001
S. faecalis	2,25±0,16	7,07±0,06	<0,001

TABLE 2. Antibodies Against E. coli 055 and Complement in Blood Serum of Germfree and Monocontaminated Guinea Pigs ($\log_2$)

Index	Animals donating serum								
	Germfree	Contaminated by B. mesentericus	P	Contaminated by B. subtilis	P	Contaminated by S. albus	P	Contaminated by S. faecalis	P
Antibodies against E. coli 055	1,00±0,33	1,30±0,09	>0,05	1,77±0,07	<0,05	5,57±0,11	<0,001	7,08±0,06	<0,001
Monocontaminated	7,07±0,10	13,69±0,08	<0,001	12,96±0,04	<0,001	9,00±0,14	<0,001	7,34±0,25	>0,05

laboratory. The sterility control was carried out by Wagner's method [8].

The choice of microorganisms for contamination was made taking into account the normal guinea pig microflora [2]. The following microorganisms were used: Bacillus mesentericus 1024, Bacillus subtilis 8236, and Streptococcus faecalis 484, obtained from the L. A. Tarasevich Government Control Institute for Medical Biological Preparations, and Staphylococcus albus 9198 obtained from the Institute of Experimental Medicine, Academy of Medical Sciences of the USSR.

The animals were contaminated by mouth on the 3rd day after receipt by administering 1 ml of a microbial suspension containing 500,000 bacterial cells. Contamination was repeated during the next 2 days. The animals were sacrificed and the number of microorganisms determined in 1 ml of the contents of the cecum by the serial dilution method. Except in the case of B. mesentericus, the number of microorganisms cultured was equivalent to 10^9 cells. The content of B. Mesentericus was 10 times greater.

The titer of antibodies against E. coli 055, pathogenic for guinea pigs, and against representatives of the intestinal microflora used for monocontamination was determined by the passive hemagglutination test (PHT) with sheep's red cells using the Takachi microtitrator by the method described in [7]. A culture of E. coli 055 B5H6, strain 27, was obtained from the L. A. Tarasevich Government Control Institute.

The presence of common antigens in E. coli 055 and the microorganisms used for contamination was established by the PHT and by Ouchterlony's double gel diffusion test [5]. The results of both tests were negative.

The complement titer was determined by the immune hemolysis of sheep's red cells test by the serial dilution method with the Takachi microtitrator [1].

The numerical results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Contamination of the germfree animals by spore-bearing aerobes (B. mesentericus and B. subtilis) had no significant effect on the titer of antibodies against these microorganisms in the serum. The difference for

B. mesentericus was not statistically significant (P > 0.05). Meanwhile S. albus and S. faecalis caused marked stimulation of antibody formation (Table 1).

Similar results also were obtained with E. coli 055 (Table 2).

As regards complement formation the spore-bearing aerobes and S. albus were most active. For S. faecalis the difference from the germfree animals was not significant (Table 2).

The results indicate that the formation of natural immunity is dependent on the microbial factor. Individual representatives of the normal microflora were found to differ in their effect on the formation of antibodies and complement. An increase in the concentration of antibodies against E. coli in the absence of common antigens between E. coli and the microorganisms used for contamination is evidence of nonspecific stimulation of the corresponding clones of antibody-forming cells by individual representatives of the normal microflora.

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KINETICS OF COLONY-FORMING ABILITY OF MOUSE BONE MARROW CELLS AFTER ADMINISTRATION OF HYDROCORTISONE

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UDC 612.419:612.6].014.46:615.357.453

The dynamics of the colony-forming and migration capacity of polypotent hematopoietic stem cells in the bone marrow of (CBA x C57BL) F_1 mice was studied after injection of hydrocortisone. The relative number of hematopoietic stem cells in the bone marrow was higher than in the control on the 3rd day after hydrocortisone injection. This increase was maximal on the 5th day after the injection. On the 8th day the number of hematopoietic stem cells was down to normal again.

KEY WORDS: polypotent stem cells; proliferation; migration; hydrocortisone.

For a long time the immunodepressive effect of glucocorticoids was explained by their cytotoxic action on the immunocompetent cells of lymphoid tissue [11]. However, recent observations have shown that cells of the thymus medulla of birds [15] and mice [7,8,10] are resistant to the action of high doses of hydrocortisone (HC). Various workers have obtained evidence to show that injection of HC into mice increases the activity of the bone marrow cells in the graft versus host reaction [10] and in response to phytohemagglutinin in vitro [12]. They consider that these effects can be attributed to migration of cortisone-resistant cells of thymus

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 9, pp. 1102-1104, September, 1976. Original article submitted March 23, 1976.