OPSONIC ACTIVITY OF THE SERUM OF GERMFREE GUINEA PIGS CONTAMINATED BY SINGLE STRAINS OF THE INTESTINAL MICROFLORA

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The effect of contamination of germfree guinea pigs by single strains of the intestinal microflora (*Bacillus mesentericus*, *Bacillus subtilis*, *Staphylococcus albus*, and *Streptococcus faecalis*), on the formation of the opsonic activity of the blood serum was studied. An increase in opsonic activity against all microorganisms was observed on the 11th day after the corresponding monocontamination, and the serum had a stimulating effect on the intracellular digestion of cells of *B. mesentericus* and *B. subtilis*. With respect to pathogenic microorganisms (*E. coli* 055) only *S. faecalis* cells were able to stimulate the opsonic activity of the serum. The results point to the existence of a connection between the composition of the microflora and the opsonic activity of the animals' blood serum. The level of this index also depends on the properties of the object of phagocytosis.

KEY WORDS: *opsonic activity of the serum*; *contamination*.

Conflicting results as regards the effect of the microbial factor on the opsonic activity of the serum have been obtained through the use of different objects of phagocytosis [3-6].

In this investigation the effect of contamination of germfree guinea pigs by single strains of the intestinal microflora on the development of the opsonic activity of the blood serum of these animals was investigated.

EXPERIMENTAL METHOD

Germfree and monocontaminated guinea pigs, aged two weeks, with five to eight animals in each group, were used. Germfree guinea pigs were obtained from the pregnant females by Caesarean section near the end of pregnancy, in a sterile isolator and were kept in accordance with the demands of germfree technology as developed in the writers' laboratory [2].

The choice of microorganisms for monocontamination was made with allowance for the composition of the normal microflora of the guinea pigs [1]. The following microorganisms were used: *Bacillus mesentericus* 1024, *Bacillus subtilis* 8236, *Streptococcus faecalis* 484, and *Staphylococcus albus* 9198. The animals were contaminated on the third day after receiving a microbial suspension by mouth in a volume of 1 ml, containing 500,000 bacterial cells. The contamination was repeated during the next two days. After sacrifice, the number of microorganisms in 1 ml of the contents of the cecum was determined by the serial dilution method. For *B. mesentericus* the number was 10^{10} cells, and for the other microorganisms 10^9.

The opsonic activity of the serum was determined from the results of phagocytosis of the microorganisms used for contamination, and also of the pathogenic strain *Escherichia coli* 055 B5H6 (strain No. 27) in it in vitro. Leukocytes (polymorphs) were obtained from the peritoneal exudate. A mixture of leukocytes from several adult (1-1.5 years) guinea pigs was used. The phagocytic test system included 0.3 ml of the test serum, 0.1 ml (10^6 cells) of a suspension of leukocytes in physiological saline, 0.1 ml ($5 \cdot 10^7$ cells) of a suspension of microorganisms in physiological saline, and 0.1 ml of a 10% suspension of homologous erythrocytes in physiological saline. Before addition of leukocytes, the microbial cells

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TABLE 1. Indices of Phagocytosis of Contaminant Microorganisms in Blood Serum of Germfree and Monocontaminated Animals (M ± m)

Index of phagocytosis	Duration of phagocytosis	Animals donating serum					
		germfree	contam. with B. mesentericus	P_1	germfree	contam. with B. subtilis	P_1
Phagocytic index	30 min	15,80±0,86	37,80±1,62	<0,001	22,20±2,12	37,80±3,21	<0,01
	2 h	23,40±0,85	35,66±3,06		31,00±1,81	36,28±2,33	
P_2		<0,001	>0,05		<0,05	>0,05	
Phagocytic number	30 min	1,29±0,06	3,20±0,15	<0,001	1,39±0,07	3,69±0,30	<0,001
	2 h	1,69±0,03	2,74±0,10		1,85±0,05	2,77±0,08	
P_2		<0,001	<0,05		<0,001	<0,05	

Index of phagocytosis	Duration of phagocytosis	Animals donating serum					
		germfree	contam. with S. albus	P_1	germfree	contam. with S. faecalis	P_1
Phagocytic index	30 min	30,40±1,63	41,50±1,15	<0,001	67,20±0,73	73,80±0,74	<0,001
	2 h	37,00±1,82	40,00±1,76		31,80±1,49	55,33±2,09	
P_2		<0,05	>0,05		<0,001	<0,001	
Phagocytic number	30 min	3,92±0,17	5,35±0,30	<0,01	6,96±0,32	20,31±1,65	<0,001
	2 h	3,37±0,26	5,15±0,19		5,11±0,08	22,13±1,43	
P_2		>0,05	>0,05		<0,001	>0,05	

Note. Here and in Table 2: P_1) significance of differences relative to germfree animals; P_2) significance of differences between indices of phagocytosis after 30 min and 2 h.

TABLE 2. Indices of Phagocytosis of *E. coli* 055 in Blood Serum of Germfree and Monocontaminated animals (M ± m)

Index of phagocytosis	Duration of phagocytosis	Animals donating serum								
		germfree	contam. with B. mesentericus	P_1	contam. with B. subtilis	P_1	contam. with S. albus	P_1	contam. with S. faecalis	P_1
Phagocytic index	30 min	37,37±2,36	33,57±1,23	>0,05	42,17±2,16	>0,05	39,60±1,63	>0,05	68,25±1,86	<0,001
	2 h	36,37±1,15	31,86±2,33		35,17±1,78		39,40±1,56		54,25±2,06	
P_2		>0,05	>0,05		<0,05		>0,05		<0,001	
Phagocytic number	30 min	1,99±0,07	1,96±0,02	>0,05	2,01±0,05	>0,05	1,97±0,04	>0,05	2,99±0,06	<0,001
	2 h	2,18±0,10	2,01±0,03		2,02±0,05		1,91±0,03		2,61±0,11	
P_2		>0,05	>0,05		>0,05		>0,05		<0,01	

were opsonized by keeping the phagocytic mixture in an incubator for 10 min. Phagocytosis was studied in films prepared from the phagocytic mixture 30 min and 2 h after addition of leukocytes. Before the 30-min sample was taken the leukocytes were washed to remove unphagocytosed bacterial cells in order to assess the state of intracellular digestion of the ingested microorganisms. A decrease in the indices of phagocytosis toward 2 h was regarded as a sign of intracellular digestion of the ingested bacteria, whereas no significant change or an increase indicated that phagocytosis was incomplete. In that case, as a rule, intracellular proliferation of the ingested microorganisms was observed. The phagocytic index and phagocytic number were used as the indices of phagocytosis.

EXPERIMENTAL RESULTS

The opsonic activity of the blood serum of the germfree guinea pigs relative to the microorganisms chosen was not identical (Table 1). It was lowest against spore-bearing aerobes, *B. mesentericus* and *B. subtilis*. Opsonic activity was strongest against *S. faecalis*. In the presence of the serum of germfree guinea pigs phagocytosis of all microorganisms except *S. faecalis* was incomplete; in the case of *B. mesentericus* and *B. subtilis* intracellular proliferation of the ingested microorganisms was observed.

During contamination of the germfree animals the opsonic activity of their serum increased against all members of the intestinal microflora and a stimulating effect of the

serum also was observed on the intracellular digestion of *B. mesentericus* and *B. subtilis*. However, relative to pathogenic microorganisms (*E. coli* 055) (Table 2), of all the contaminating microorganisms chosen, the only one able to stimulate opsonic activity was *S. faecalis*.

The results indicate that there is a connection between the composition of the microflora and the opsonic activity of the blood serum of animals. The actual value of this index also depends on the properties of the object phagocytosed.

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IMMUNE RESPONSE OF MICE OF VARIOUS INBRED LINES TO *Clostridium oedematiens* TOXOID

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Mice of various inbred lines were immunized intradermally with *Clostridium oedematiens* α toxoid. The immunization was repeated 30 days later. The titer of antibodies against toxoid was determined by the passive hemagglutination test in the blood of the mice 20 and 30 days after the first and 10 days after the second immunization. The maximal response to primary immunization was recorded in C3H mice, the minimal in DBA/2 mice, with a more than 30-fold difference. The remaining tested lines of mice (A, CBA, BALB/c, AKR, C57BR) occupied an intermediate position. After the second immunization the differences were reduced. The existence of genetic control of the immune response to this particular antigen is postulated in mice.

KEY WORDS: Clostridium oedematiens *toxoid*; *antibody formation*; *genetic control*.

As has been shown repeatedly, ability to give an immune response to concrete antigens is under genetic control in animals and man [2, 6]. The separate genes controlling the immune response have now been identified. These genes may be linked with the main system of genes determining histocompatibility in mice (H-2), guinea pigs (GPL-1) man (HLA), etc., or they are not linked with it (for example, the IR-3 locus in mice). The search for highly immune antigens with the minimal number of antigenic determinants on the molecule is being pursued intensively, for it is only by the use of such antigens that any further elucidation of the mechanisms of control of the immune response will be achieved.

In this investigation antibody formation was studied in mice of different lines during immunization with *Clostridium oedematiens* toxoid.

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