IMMUNOGLOBULINS OF VARIOUS CLASSES IN THE MOUSE INTESTINE AFTER ORAL CONTAMINATION WITH Escherichia coli

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Changes in the levels of the various classes of immunoglobulins in the intestinal contents of NMRI/HAN mice were studied after oral contamination of the animals with Escherichia coli (serologic group 055). The quantity of IgA in the intestinal contents was found to be increased and that of IgG reduced 20 days after administration of the bacteria. It is concluded that immunoglobulin synthesis in the intestine (especially that of IgA) is one of the first protective responses to microbial action in intestinal infections.

KEY WORDS: immunoglobulins; immunoglobulin synthesis in the intestine.

In recent years attention has been redirected to the role of antibodies in the lumen of the intestine. Experiments on germ-free pigs have shown that the presence of corresponding antibodies in the intestine protects piglets against infection with colibacillosis [7, 12, 13]. If germ-free mice were immunized with salmonella antigens, antibodies also were found in the intestine [3]. Antibodies against viruses are also present in the intestinal contents [2].

The titer of intestinal antibodies is much higher after oral administration of an antigen [9]. In experiments on rats, rabbits, and piglets, migration of antibodies was found from the serum into the intestinal lumen [14] and from the intestinal lumen into the blood serum [8, 12]. The most likely source of IgA is considered to be the subepithelial plasma cells of the intestinal mucous membrane [4]. The IgA are the predominant immunoglobulins in the mouse intestine [3]. The presence of other classes of immunoglobulins in secretions of the intestinal mucous membranes likewise cannot be ruled out [1], although the IgA activity in the mouse intestine was found to be 8 times higher than that of IgM and 25 times higher than that of IgG [14]. Antiviral intestinal antibodies were also associated mainly with IgA [2].

The object of the present investigation was to study changes in the levels of the various classes of immunoglobulins in the intestinal contents of mice after immunization of the animals with E. coli cells (serologic group 055).

EXPERIMENTAL METHOD

Sexually mature mice of line NMRI/HAN were used. The animals were contaminated with a 24-h culture of E. coli 055 given by mouth in two doses, each of 10 million bacterial cells, separated by an interval of 10 days. The mice of the control group were kept under identical conditions without contamination. Each of the experimental and control groups contained 20 mice.

The animals were killed 20 days after the last dose of E. coli and the intestine was carefully removed. Sterile physiological saline in a volume of 2 ml was injected from a syringe into the small intes-

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TABLE 1. Accumulation of Immunoglobulins in Intestine of Immunized and Control Mice

Mice	No. of mice	Immunoglobulins in secretions of intestinal mucous membrane (diam. of precipitation zone in cm)		
		IgA	IgM	IgG
Immunized	20	0,84±0,01	0,74±0,04	0,45±0,01
Control	20	0,76±0,02	0,65±0,02	0,51±0,01
P		<0,01	>0,05	<0,01

tine and the contents of the intestine were propelled by gentle massage. Then, by means of a rubber roller, the contents of the small intestine were gently expelled into centrifuged tubes. The material was preserved with 0.05% sodium azide solution.

To obtain the supernatant the material was centrifuged in a refrigeration centrifuge at 15,000 rpm for 15 min at 4°C, after which the supernatant was withdrawn into a pipet.

The whole procedure of obtaining the material occupied not more than 1-1.5 h and was carried out on ice. The material was used in the agglutination test with a cell suspension of E. coli 055 obtained by treating 24-h agar cultures with 0.5% formalin solution and then washing with phosphate buffer, pH 7.4.

Changes in the levels of the various classes of immunoglobulins in the intestinal contents during the action of E. coli cells on the intestinal wall were studied by the radial immunodiffusion method [5]. Monospecific sera against mouse immunoglobulins (anti-IgA, IgM, and IgG) were obtained from the Department of Immunology (Director, Professor J. Sterzl), Institute of Microbiology, Czechoslovak Academy of Sciences, The antisera were used in the radial immunodiffusion test in a dilution of 1:4.

Agar (Difco), mixed with antisera against IgA, IgM, and IgG, respectively, was poured into a chamber constructed on glass slides. Standard wells made in the agar were filled with the intestinal contents for study, in a dilution of 1:2. The slides were incubated at room temperature for 24 h. After the end of immunodiffusion the slides were stained with amido black and the diameters of the precipitation zones were measured with a ruler. The mean values of three measurements were obtained.

EXPERIMENTAL RESULTS AND DISCUSSION

The agglutination test revealed no specific antibodies (agglutinins) against E. coli 055 in the intestinal contents, possibly because of the very slight accumulation of antibodies in the intestinal contents in immunization by this method.

The immunodiffusion test in gel revealed immunoglobulins of all three classes in the intestinal contents of the immunized and control mice. The results of the determinations of the various classes of immunoglobulins in the mouse intestine are given in Table 1. Clearly the quantity of IgA and IgM in the intestinal contents of the mice increase after oral immunization with E. coli, whereas the content of IgG fell below the control level.

The increase in the IgA titer after oral immunization can be explained on the grounds that the intestine of mice and other species of animals contains large numbers of plasma cells, that synthesize this immunoglobulin [4, 14]. Synthesis of IgM is not thereby ruled out [4], and this also was observed in the experiment described. The experimental data agree with clinical investigations. In patients with shigella infections there is a considerable increase in the IgA content in the intestine, but no significant change in the concentrations of IgM and IgG [10].

In man IgA is present in the saliva, gastric juice, bile, pancreatic juice, the contents of the jejunum, and filtrates of the stools [11]. There is evidence that cells producing IgA possess immunologic memory [6] and that the synthesis of IgA takes place in situ, unlike that of other globulins, which evidently arise from the serum [1].

It can be concluded from data in the literature and the results of this experiment that the synthesis of immunoglobulins (particularly IgA) in the intestine is one of the first protective responses of the organism to microbial action in intestinal infections.

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