IMMUNOGLOBULINS A AND G IN INTESTINAL SECRETION OF CONVENTIONAL AND GERMFREE RATS

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The study of the mechanism of formation of local immunity is an urgent theoretical task related to the solution of problems in the prevention and treatment of enteric infections.

Among the factors responsible for local defense of the body against infectious agents, according to data in the literature an important role is played by secretory IgA (SIgA) [1, 2, 8, 10-12]. Meanwhile there are reports that IgA antibodies have no bactericidal activity and even that they inhibit the bactericidal activity of IgG and IgM antibodies [7, 13, 14].

The object of this investigation was to determine the effect of the normal microflora and of agents of enteric infections (*Shigella flexneri*) on the immunoglobulin level in rat intestinal secretions.

EXPERIMENTAL METHOD

Inbred CDF(F344)CRL rats, aged 3 months, were used and were divided into the following groups: germfree (30 animals), conventional (30), and germfree, infected with *Sh. flexneri* (18). The methods of rearing the germfree animals and of bacteriological control were described previously [5]. Germfree rats were infected with a suspension of an 18 h culture of *Sh. flexneri* 2^a, strain No. 516, in a dose of 1×10^9 bacterial cells in 0.5 ml per animal, which was added to the food or injected into the stomach through a tube. Specific antibodies against *Sh. flexneri* were determined in the passive hemagglutination test on a Takichi microtitrator. The S-IgA were isolated from intestinal secretion of conventional rats [6]. Antiserum against SIgA was obtained in the same way as that to IgG^2 [3]. Both antisera were used to determine immunoglobulins in intestinal secretions and blood sera of rats by the radial immunodiffusion (RID) test [9].

EXPERIMENTAL RESULTS

The antiserum produced as described above revealed one protein in the intestinal secretion, colostrum, and blood serum of adult rats with electrophoretic mobility corresponding to SIgA or serum IgA (Fig. 1); the latter is not present in newborn rats in the first 3 weeks of life [4]. These findings confirm that the antiserum was in fact produced against SIgA.

Preliminary experiments showed that intensive destruction of immunoglobulin molecules takes place in the large intestine. Since the intensity of this process was much greater in conventional rats, it can be concluded that the intestinal microflora plays a more important role in this process than the proteolytic enzymes of the digestive tract. Accordingly, secretion from the rat jejunum was used for quantitative assay of SIgA and IgG.

The results showed that germfree rats have no less SIgA than conventional rats (Fig. 2a). Consequently, the intestinal microflora has no significant effect on stimulation of synthesis of antibodies of this immunoglobulin class. The decisive role in this case is probably played by food antigens and, in particular, by protein feeding. In the present vestigation the rats received a diet rich in animal protein (24% of casein), whereas in studies cited above [8, 10] the germfree mice were kept on a diet containing mainly vegetable proteins, or on a water-soluble nonantigenic diet. Under these circumstances SIgA was absent from the secretion and intestinal contents of the germfree mice.

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TABLE 1. Determination of Specific Antibodies in Blood Serum and Intestinal Secretion of Germfree Rats Infected with *Sh. flexneri*

Test material	Time of investigation, days	Antibody titer, $\log_2$										
		2	3	4	5	6	7	8	9	10	11	12
Blood serum	7	0	0	0	0	0	0	0	0	0	0	0
	14	0	0	0	0	0	0	0	0	0	0	0
	21	0	0	0	0	0	0	0	0	0	0	0
	42	+	+	+	0	0	0	0	0	0	0	0
Intestinal secretion	7	+	+	+	+	0	0	0	0	0	0	0
	14	+	+	+	+	+	+	0	0	0	0	0
	21	+	+	+	+	+	+	+	+	+	0	0
	42	+	+	+	+	+	+	+	+	+	+	+

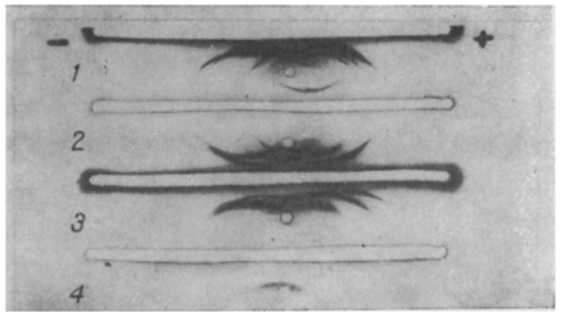

Fig. 1. Immunoelectrophoresis in agar. Wells contain: 1) blood serum from rat aged 6 months, 2) blood serum from rats aged 7 days, 3) rat colostrum, 4) secretion of small intestine from rats aged 6 months. Gutters contain: 1 and 3) antiserum against rat blood serum proteins, 2 and 4) antiserum against rat SIgA.

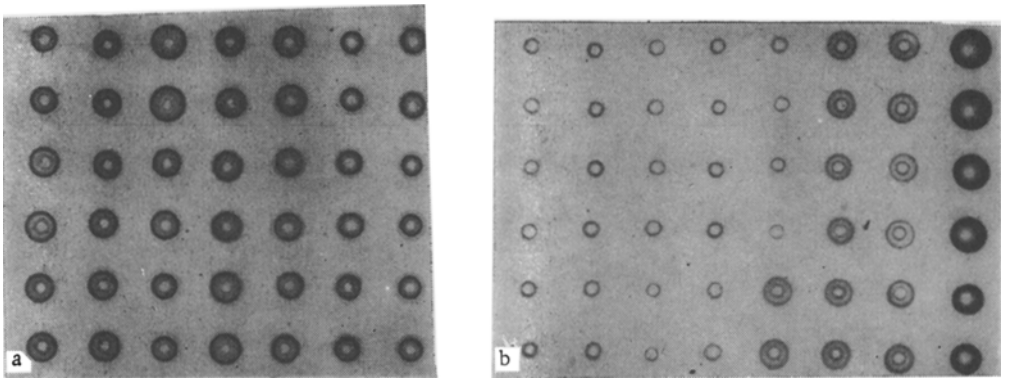

Fig. 2. Radial immunodiffusion: a) agar contains antiserum against rat SIgA in dilution of 1:20; b) agar contains antiserum against rat IgG in same dilution. Wells contain whole secretion from rat jejunum (2 µl in each case): 1-28) from germfree rats, 29-42) from conventional rats (counting from top to bottom). 2b) 43-48) pooled rat serum in dilution of 1:10 in volumes of 2.0, 1.5, and 1.0 µl, respectively.

A different picture was observed when IgG was determined (Fig. 2b). IgG was virtually absent from the intestinal secretion of the germfree rats. The character of the precipitate formed in the RID by IgG^2 from blood serum and intestinal secretion with antiserum against IgG^2 from rat serum was different, and the explanation cannot be quantitative, for the blood serum of conventional rats contains 6-7 times more IgG^2 than that of germfree rats [3], although this is reflected only in the size of the rings in the RID. When blood serum and intestinal secretion were tested in Ouchterlony's precipitation test against anti-IgG^2 serum, the serum immunoglobulin formed a "spur," i.e., it had an additional antigenic determinant. Differences in the immunoelectrophoretic mobility of IgG from human blood and colostrum have been reported in the literature [14].

The strain of *Sh. flexneri* used to infect the animals could be isolated from the feces after the 2nd day in a concentration of 10^6 cells/g. At autopsy and quantitative determination of *Sh. flexneri* cells in different parts of the gastrointestinal tract the results were as follows: small intestine 10^6 cells/g contents, cecum 10^7-10^8 cells/g, feces 10^6 cells/g. This number of microorganisms remained stable during observations up to the end of 3 months.

The IgA level in the blood serum and intestinal secretion of the rats 6 weeks after infection was the same as previously, but traces of IgG began to appear in the intestinal

secretion as early as after 1 week. Toward the end of the investigation their titer reached 20% of that in the blood serum. Meanwhile, 1 week after infection specific antibodies against *Sh. flexneri* were found in the intestinal secretion, and by the 21st day their titer exceeded 1:4096. Antibodies appeared in the serum at the 6th week, in low titers (1:16, 1:32; Table 1). Consequently, synthesis of specific antibodies found in the intestinal secretion of germfree rats infected with *Sh. flexneri* began sooner in the intestinal wall, i.e., the local immune response was more marked than the generalized response. However, persistence of *Sh. flexneri* in the intestine of germfree rats did not come to an end, but a clincally healthy bacterial carrier state developed in the immune animal.

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SYNTHESIS OF A HIGH-CAPACITY IMMUNOSORBENT BASED ON A CELLULOSE SUSPENSION

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Antibodies and antigens fixed to an insoluble base (immunosorbents — IS) are now widely used in immunology and molecular biology [2, 5, 7]. To increase the total surface on which interaction takes place between antigen and antibody molecules, the writers previously used a very fine suspension of cellulose ester, obtained by reprecipitating cellulose from cuprammonium solution, in order to prepare IS [3, 4].

In this paper a simple method of obtained IS with higher capacity and with a better ratio between the quantities of fixed antigen and antibodies bound to it, is suggested. For this purpose the protein was bound to the cellulose suspension through aldehyde groups [6, 9].

Cellulose powder* was used as the insoluble base for IS. The cellulose was converted into a finely dispersed suspension by reprecipitation from cuprammonium solution by the method described previously [3], the difference being that cellulose and not its ester was taken. The cellulose was oxidized with sodium periodate ($NaIO_4$, from Reanal, Hungary) [6]. The content of reducing aldehyde groups was determined by Szabolks' method [10]. Rabbit γ-globulin (RGG) (from Calbiochem, USA) was used as antigen, and donkey antiserum against RGG, produced by the N. F. Gamaleya Institute of Epidemiology and Microbiology, as antiserum. To

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