A STUDY OF THE PLASMOCYTIC REACTION IN ENTERAL IMMUNIZATION AGAINST TYPHOID

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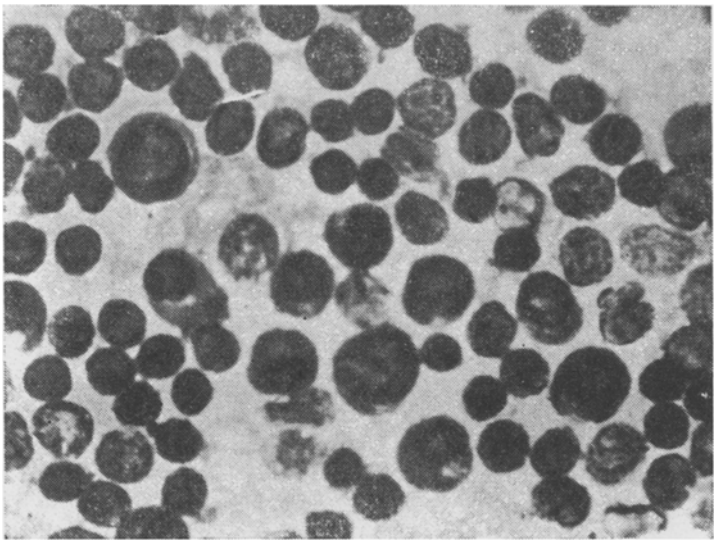
The problem of the site of antibody formation after parental injection of various antibodies has been discussed in a great many papers [1-4, 6-13]. It has been established that plasmocytic cells of the lymph nodes are responsible for the production of antibody following enteral injection of antigen. However, except for a single case [5], the plasmocytic reaction associated with enteral immunization has received no attention in the literature.

We have studied the plasmocytic reaction of the lymph nodes following parental immunization of mice against typhoid. We have also carried out a parallel series of investigations into the general resistance of immunized animals to infection by cultures of typhoid bacteria.

EXPERIMENTAL METHODS

Mice were immunized enteral injections of live E. coli culture 5396/38, containing Vi-antigen and with Vi-antigen isolated from the particular culture by the Webster-Lund method, using a modification described by Konikov and Klyucheva. The immunization was carried out using 2 cycles of 3 day injections one after the other and an interval between each cycle of 10 days. The immunization substrate was injected through the mouth of the



Impression preparation of a mouse lymph node from an animal given parental immunization with E. coli 5395/38, using the threefold, bicyclic method. Photomicrograph. Stained Romanowsky-Giemsa. Immersion ocular × 10, objective × 95.

TABLE 1. Plasmocytic Reaction and Resistance of Mice Given Enteral Immunization Against Typhoid (1st experiment)

Immunizing substance	Resistance		Plasmocytic reaction			
	Number of animals	Number surviving	4th day after 1st cycle		15th day after 2nd cycle	
			plasmoblasts	plasmocytes	plasmoblasts	plasmocytes
Live culture E. coli 5396/38	28	20 (71.4%)	102	38	31	120
Vi-coli antigen (0.1 mg per 0.1 ml)	24	9 (37.7%)	137	53	40	22
Control plasmocytic reaction (non-immunized mice)	—	—	15	4	17	6
Control of culture S. typhi Ty_2	10	0	—	—	—	—

syringe with a blunt needle. The live culture was used at a dosage of 10 million microbial bodies per 0.1 ml. Immunization with the antigen was carried out at doses of 0.1 and 1 mg per 0.1 ml.

Fifteen days after the last injection of the 2nd cycle the animals were infected with a virulent S. typhi culture Ty_2 using an intra-abdominal injection of 50 million microbial bodies in 0.5 ml physiological saline, which corresponded to 1 Dcl.

TABLE 2. Plasmocytic Reaction and Resistance of Mice Given Enteral Immunization Against Typhoid (2nd Experiment)

Immunizing substance	Resistance		Plasmocytic reaction							
	No. of animals	Number surviving	4th day after 1st cycle				15th day after 2nd cycle			
			para-aortal lymph node		mesenterial		para-aortal lymph node		mesenterial	
			plasmoblasts	plasmocytes	plasmoblasts	plasmocytes	plasmoblasts	plasmocytes	plasmoblasts	plasmocytes
Live culture E. coli 5396/38	16	14 (87.5%)	78	15	83	19	51	120	35	140
Vi-coli-antigen (1 mg per 0.1 ml)	35	29 (82.8%)	69	20	54	12	73	67	52	117
Vi-coli-antigen (0.1 mg per 0.1 ml)	33	11 (33%)	—	—	—	—	25	31	33	41
Control plasmocytic reaction	—	—	20	7	13	5	19	17	9	7
Control of culture S. typhi Ty_2	10	0	—	—	—	—	—	—	—	—

In the first experiment we studied the reaction of the para-aortal and mesenterial nodes. Preparations of the lymph nodes were investigated 4 days after the commencement of immunization and on the 15th day after the 2nd cycle. They were stained with Romanowsky-Giemsa. The plasmocytic cells in 50 fields of the microscope were counted, making a differential count of plasmoblasts and plasmocytes. The final result was expressed as the mean of the counts made from the lymph nodes removed from 3 mice.

Together with our examination of the experimental mice, we investigated the corresponding lymph nodes from non-immunized, control mice on each occasion.

Furthermore, we made preparations of lymph nodes stained with Brash's methyl green-pyronin in order to demonstrate the presence of ribonucleic acid in the plasmocytes. As a control, some of the preparations were treated with ribonuclease before staining.

EXPERIMENTAL RESULTS

In Tables 1 and 2 is set out data relating to our study of the general resistance of mice immunized enterally with various preparations, together with the nature of the plasmocytic reaction of the lymph nodes.

We have established that there is a significant plasmocytic reaction of the lymph nodes in immunized animals as compared with the mice of control groups. Analysis of results obtained by us revealed that after the first immunization cycle juvenile forms of the plasmocytic cell group predominated. In microscopic preparations stained with Romanowsky-Giemsa, we often encountered plasmoblasts—large cells with a round nucleus and a narrow ring of blue cytoplasm (c f. figure). At the end of the immunization process the number of juvenile cells had undergone a considerable reduction with a corresponding increase in the number of mature forms—the plasmocytes. At this time the field of the microscope was dominated by small cells, each with a small compact nucleus situated eccentrically in the blue cytoplasm which appeared as a translucent zone around the nucleus (c f. figure).

Preparations of lymph nodes from control animals possessed few cells of the plasmocytic type.

We found the most marked plasmocytic reaction after immunization with a live culture of coli bacteria containing Vi-antigen. On the 15th day after immunization the total of plasmoblast and plasmocyte cells amounted to 175 in 50 microscope fields. The comparable figure for lymph nodes of control animals was 16.

On staining a portion of the lymph node preparations with methyl green-pyronin using Brash's technique we found a large RNA content to the plasmocyte cells of lymph nodes from immunized animals and this suggested that active antibody synthesis was going on. As controls we stained preparations which had previously been treated with ribonuclease. No specific coloration was noticed among the controls. Consequently, we could assume that the red coloration which developed in the experimental preparations was specific for ribonucleic acid.

The results obtained (c f. Tables 1 and 2) indicate a correlation between the general resistance of the immunized mice and the intensity of the plasmocytic reaction. Those experiments in which there was the highest percentage survival among the animals were also the experiments in which the most intense plasmocytic reaction was observed.

After immunization with living E. coli culture containing Vi-antigen followed by infection with 1 Dcl typhoid culture, the survival rate of the mice concerned was 70-80%. An intense plasmocytic reaction was noticed among such immunized animals whereas among control animals the number of cells of the plasmocytic type was considerably less. On immunizing with small doses of Vi-antigen (0.1 mg) followed by infection with typhoid, only 33% of the animals survived and in this group the plasmocytic reaction was feeble. Immunization with an increased dose of Vi-antigen (1 mg) produced a survival of 82.8%. Associated with this increase in the survival rate we observed a corresponding increase in the intensity of the plasmocytic reaction.

SUMMARY

The count of the plasmocytic series cells increased in the para-aortic and mesenteric lymph nodes during enteral immunization of mice with live E. coli culture, containing Vi-antigen, as well as with the Vi-antigen isolated from the mentioned culture. There was an increase in the number of juvenile forms of the plasmocytic series at the beginning of the immunization process; the number of mature forms exhibited a marked rise by the end of immunization. Considerable accumulation of ribonucleic acid was seen in the cells of the plasmocytic series in the lymph nodes of the immunized animals. Relation was demonstrated between the general resistance indices in the immunized animals following typhoid culture infection, and the intensity of plasmocytic reaction.

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