THE STUDY OF THE IMMUNOLOGICAL FUNCTIONS OF LYMPHOID TISSUE BY THE METHOD OF CELL TRANSFER. REPORT 2. THE ABILITY OF SPLENIC CELLS FROM IMMUNIZED MICE TO PROTECT RECIPIENTS AGAINST EXPERIMENTAL INFECTION

N. A. Kraskina and V. I. Levenson
Division of Immunology (Head, Professor M. P. Pokrovskaya), Moscow Research Institute of Epidemiology and Microbiology (Director, S. I. Didenko)
(Presented by Active Member AMN SSSR the late V. L. Troitskii)
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The ability of the cells of the lymphoid tissue from an immune animal to synthesize specific antibodies after transplantation in an unimmunized recipient has been demonstrated by several workers [4, 6, 7]. Far less research has been done on the problem of the ability of these cells to protect the unimmunized recipient against the corresponding infections. The transplantation of lymphoid cells from donors immunized with pneumococcal polysaccharide protects the recipients against pneumococcal infection [5]. The results described in Report 1 showed that the transfer of splenic cells from mice immunized with a typhoid Vi-antigen preparation leads to the accumulation of Vi-antibodies in adult, nonirradiated recipients, which must be attributed to the function of the transplanted cells.

In the present research we sought to discover whether the transfer of cells from "immune" lymphoid tissue can give protection against typhoid infection and to determine the degree of protection conferred.

EXPERIMENTAL METHOD

The experiments were conducted on 458 albino mice, weighing 16-18 g. The donor mice were immunized with a chemical preparation of Vi-antigen from typhoid microorganisms. The preparation was injected intravenously in a dose of 1 μg. The Vi-antibodies in the sera of the animals were determined by the passive hemagglutination reaction. The spleen was extracted from the donor mice 3 days after injection of the antigen. The technique of preparation of the cell suspension and of its administration to the recipients is described in the previous report.

The recipient mice were infected 2 days after injection of the cell material. An 18 hr agar culture of Salmonella typhi Ty^2 was used to inject the animals, and was injected intraperitoneally in doses of 25, 100 and 400 million bacterial cells. From the results of infection, the LD_{50} of the S. typhi culture for the mice of the different experimental groups was determined and compared with that for the control animals receiving no treatment before infection. The value of LD_{50} was calculated by I. P. Ashmarin's method [1]. Furthermore, for each group we determined the index of efficacy (IE) of immunization [3], using the equation:

$$IE = \frac{LD_{50} \text{ of S. typhi culture for immunized mice}}{LD_{50} \text{ of S. typhi culture for control mice}}.$$

EXPERIMENTAL RESULTS

The principal results obtained from three experiments are shown in Table 1 and in the figure.

The experimental animals were divided into four groups. The mice of the first group were the experimental animals: they received a suspension of the splenic cells of immunized donors ("immune" spleen). The mice of the remaining groups were controls. The mice of the second group received the same cell suspension, but after it had been heated to 56° for 30 min. As we showed in our previous report, this treatment destroys the cells of lymphoid tissue but does not affect the immunological activity of the Vi-antigen if it is present in the cell suspension. The mice of the third group received a suspension of splenic cells from unimmunized donors ("normal" spleen). The animals of the fourth group were injected with the serum of immunized donor mice, the spleens of which were used for injection into the recipients of the first group. In all groups, injections were made intraperitoneally in 0.5 ml doses.

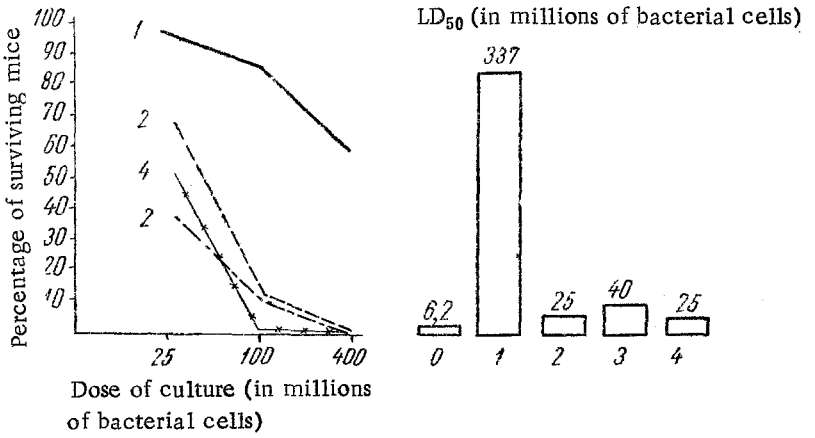
TABLE 1. Degree of Protection of Recipient Mice on the 2nd Day after Transfer of Splenic Cells

Material injected into mice	Experiment No.	Dose of culture injected (millions of bacterial cells)					LD_{50} (millions of bacterial cells)	IE	Titer of antibodies in recipient
		6.3	12.5	25	100	400			
		survived after infection[1]							
Cells of immune spleen	1	–	–	4/5	5/5	5/5	603	96	1:640
	2	–	–	10/10	7/10	2/10	174	29	1:160
	3	–	–	4/4	5/5	4/4	>600	>50	1:640
Total		–	–	18/19	17/20	11/19	337	42	1:385
Heated cells of immune spleen	2	–	–	4/10	1/9	0/10	25	4	0
Cells of normal spleen	2	–	–	5/8	0/8	0/8	25	4	0
	3	–	–	6/8	2/8	0/8	50	4.2	0
	Pooled results	–	–	11/16	2/16	0/16	40	4	0
Immune serum[2]	1	–	–	5/5	0/5	0/5	50	8	0
	2	–	–	3/10	0/10	0/10	19	3	1:4
	Pooled results	–	–	8/15	0/15	0/15	25	4.4	1:2
Control	1	1/4	1/4	0/4	–	–	6.3	–	–
	2	2/5	0/4	0/4	–	–	6.0	–	–
	3	9/10	5/10	0/10	–	–	11.7	–	–

[1] - Numerator - number of surviving mice; denominator - number of mice infected.

[2] - Serum with a titer of 1:2560 was injected intraperitoneally in a dose of 0.1 ml 48 hr before infection.

Five mice from each group were sacrificed and used for determination of the serum antibodies. The remaining animals were infected with a culture of S. typhi 48 hr after injection of the cell material. The control animals were infected at the same time; the LD_{50} of the S. typhi culture for the latter varied in different experiments from 6 to 11.7 million bacterial cells. A dose of 25 million bacterial cells was lethal for all the control mice.



Degree of protection of mice against injection in experiments with cell transfer. 0) Control; 1) recipients of cells of "immune" spleen; 2) recipients of heated cells of "immune" spleen; 3) recipients of cells of "normal" spleen; 4) mice receiving 0.1 ml of immune serum intraperitoneally.

The results of these experiments (Table 1) show that injection of a suspension of splenic cells from immune donors into nonimmune mice conferred a high degree of protection against typhoid infection on the recipients when the intensity of immunity was tested on the 2nd day after transplantation, at the time of maximal antibody formation. In fact, the transfer of cells of the "immune" spleen protected nearly all the animals against infection with a dose of 25 million bacterial cells, 85% of animals against a dose of 100 million cells, and 58% of animals against a very large dose – 400 million bacterial cells (pooled data). LD_{50} of the S. typhi culture for the recipients receiving a suspension of "immune" spleen was 337 million bacterial cells, compared with a value of 8 million bacterial cells in the control groups. The index of efficacy of the transfer of immune cells was 42. In other words, the cells of the "immune" spleen raised the resistance of the unimmunized mice to S. typhi 42 times.

Infection of the animals of the remaining groups showed that they received rather better protection than the control mice. In all these groups 40-70% of the mice survived a dose of 25 million bacterial cells, although nearly all the mice died after infection with doses of 100 and 400 million bacterial cells. LD_{50} in all these groups was 25-40 million bacterial cells, and IE was 4 (Table 1). The increase in the resistance of the animals receiving heated cells or cells of "normal" spleen was evidently nonspecific in character and was associated with the inflammatory process in the peritoneal cavity caused by injection of the cell material. The degree of protection of the mice receiving immune serum may be explained both by the above-mentioned nonspecific process and by the preventive action of the serum; in this particular study there is no evidence to clarify this problem.

Comparison of the data obtained in the experimental groups shows that the protection conferred on the mice after the experimental transfer of "immune" cells is not due to active immunization by the antigen present in the transplanted material (control with heated suspension). Nor can the protection be attributed to the nonspecific action of the splenic cells (control with injection of a suspension of "normal" spleen) or to the preventive action of the antibodies present ready-made in the transplanted material. It may be supposed that this protection is conferred as a result of the specific immunological activity of the cell elements of the "immune" lymphoid tissue, which can be exercised by these elements when transferred to a nonimmune recipient. It is probable that the protection conferred by the transplanted cells is closely related to the formation by them of specific antibodies.

In this connection it is important to discover whether a high degree of protection against typhoid infection can be conferred on mice by producing a high concentration of Vi-antibodies in their serum. An experiment was performed in which the intensity of the immunity in mice receiving cells of "immune" spleen was compared with that in passively immunized mice (Table 2).

The experimental animals were divided into two groups. The animals of the first group received a suspension of cells of "immune" spleen by the method described; 48 hr after the transfer they were infected with a culture of S. typhi. The animals of the second group received an intravenous injection of 0.3 ml of homologous Vi-serum with a titer of 1:10,240 1 hr before infection. The results in Table 2 show that the animals of both groups had a high degree of protection. LD_{50} for the passively immunized mice was 263 million bacterial cells, and for the recipients of "immune" splenic cells 600 million bacterial cells (compared with 11.7 million bacterial cells for the controls). The high level of protection corresponded to the high titer of Vi-antibodies in both groups of animals. The somewhat higher degree of immunity in the recipients of the immune cells may be attributed to the nonspecific protective action of the inflammatory process in the peritoneal cavity.

TABLE 2. Intensity of Passive Immunity in Mice

Group	Dose of culture injected (millions of bacterial cells)					LD_{50} (millions of bacterial cells)	Titer of antibodies in serum
	6.3	12.5	25	100	400		
	survived after injection[1]						
Recipients of cells of "immune" spleen	–	–	4/4	5/5	4/4	600	1:640
Passively immunized mice[2]	–	–	10/10	10/10	2/10	263	1:1280
Control	9/10	5/10	0/10	–	–	11.7	0

[1] - Numerator – number of surviving mice; denominator – number of mice infected.

[2] - Serum with a titer of 1:10,240 injected intraperitoneally 1 h before infection in a dose of 0.3 ml.

These results demonstrate that the transplantation of lymphoid tissue cells from an immunized donor to a non-immune recipient is not only accompanied by the accumulation of antibodies in the latter's serum, but also confers protection on the recipient against typhoid infection. It seems probable that a causal connection exists between these two phenomena, and that the high degree of protection resulting from the transplantation of immune lymphoid tissue cells is due to the appearance of antibodies in the organism of the recipient. In this case the high degree of protection conferred on the recipients may be used as an index of the functional integrity of the Vi-antibodies formed by the transplanted cells. It cannot, of course, be excluded that other, as yet undiscovered, mechanisms may play a part in the formation of immunity following transplantation of cells.

As a result of a detailed comparative study of the mechanisms of the immunity arising after active immunization and after the transplantation of lymphoid tissue, some light has been shed on the immunological powers of this tissue, and it has been demonstrated how the manifestations of artificial immunity are associated with the other systems and tissues of the body.

SUMMARY

Homotransplantation of lymph cells of mice immunized with the chemically purified Vi-antigen provides a high degree of protection from infection with typhoid bacilli on the 2nd day after the transfer of the cells. As demonstrated, this protection is bestowed by the action of the cells transferred.

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