# A STUDY OF THE IMMUNOLOGICAL FUNCTION OF LYMPHOID TISSUE AFTER CELL TRANSFER

## I. THE FORMATION OF ANTIBODIES BY SPLEEN CELLS FROM IMMUNE MICE AFTER TRANSPLANTATION INTO ADULT UNIRRADIATED RECIPIENTS

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It has been established that cells of the lyphoid tissue transplanted from an immune donor to a nonimmune recipient retain the ability to form antibodies. The method of cell transfer has recently been performed with success in a number of laboratories where the experimental analysis of humoral immunity and immunological significance of different organs are being studied [2, 3, 4, 6, 7]. The sensitivity of this method and the significance of the results obtained are dependent on the intensity of antibody formation in the transplanted cells. Therefore the majority of workers use as donor animals those which have been immunized many times with large doses of antigen. This creates additional difficulties in experimental design in that the presence of significant amounts of antigen in the transplanted material evokes an active reaction of antibody formation in the recipient. This difficulty can be overcome by using an irradiated or newborn animal as the recipient as they are incapable of forming antibodies. However in this instance the transferred cells are situated in conditions not physiologic in the normal immunologically mature animal [4, 5].

In the present study an experimental model is described for studying immunologic function of lymphoid tissue based on homotransplantation into normal adult recipients of spleen cells of mice which had been immunized once with a chemical preparation of Vi antigen of typhoid.

## EXPERIMENTAL METHODS

1308 white mice were used in the experiments. The Vi antigen used for immunizing the donors was prepared from typhoid bacilli by the method of Webster and Landy [1]. One microgram of Vi antigen in 0.2 ml of saline was administered once intravenously. Three days later (the first day of antibody production) the donors were killed; the titer of Vi antibody was determined in the serum and in spleen extracts. The cell suspension from the donor spleens was prepared in Hanks medium (pH 7.0-7.2). The cells were rapidly transferred giving the suspension intraperitoneally to nonimmunized recipients in a 0.5 ml volume.

Each recipient received cells from a single donor (spleen weight 75 to 125 mg). Calculations indicated that from 1 mg of splenic tissue about 1 million cells are obtained; thus each recipient received 75-125 million cells. For the determination of Vi antibody titers in the recipient as well as for the titration of sera and splenic extracts of the donors a modification of the hemagglutination method of Landy [2] was used. Human erythrocytes of group 0 were sensitized with the Vi preparation.

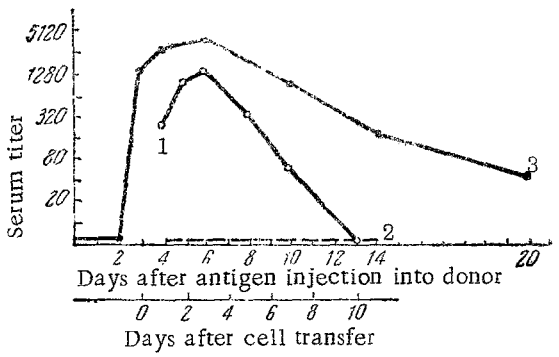
Two days after the intravenous inoculation of Vi antigen into donor mice no titer was found in the sera. On the third day, i.e., at the time of transfer, the Vi titer in the donor sera was 1:1280 to 1:2560. Antibody was also detected in the spleen on the third day and at the time of transfer the titers in the splenic extracts was 1:320-1:640.

In the first series of experiments it was shown that two days after transplantation of spleen cells from immune donors antibodies were detectable in the sera of the recipients in a titer of 1:1280 (see table).

These observations permit different explanations as the appearance of antibody in the recipients can be conditioned by: 1) the injection into the recipient of preformed antibody contained in the transferred material (passive immunization); 2) immunologic reaction of the recipient to Vi antigen fixed to the donor spleen cells (active immunization; 3) formation of antibody by transferred cells. The first hypothesis is refuted by the experiments in which mice were given serum from an immune donor in the same quantity which corresponded to the amount in the spleen tissue – 0.08 ml. Despite the fact that the antibody content in this serum was greater than in the transplanted cells they were detected after 48 h in the recipient's serum in minimal titers or not detected at all (see table).

The Amount of Vi Antibody in the Sera of the Recipient Mice 48 h after Transfer of Immune Spleen Cells

| Experiment No. | Transfer of immune cells | | Control 1: active immunization with Vi antigen | | Control 2: passive immunization with Vi serum (0.08 ml) | |
|---|---|---|---|---|---|---|
| | no. of recipients | antibody titer in recipients | no. of animals | titer of antibody | no. of animals | titer of antibody |
| 1 | 5 | 1:640 | 21 | 0 | 5 | 0 |
| 2 | 6 | 1:1280 | 15 | 0 | 5 | 1:20 |
| 3 | 10 | 1:1280 | 24 | 0 | 9 | 0 |
| 4 | 6 | 1:1280 | – | – | 6 | 1:10 |
| 5 | 20 | 1:1280 | – | – | 6 | 0 |
| Total | 47 | 1:1280 | 60 | 0 | 31 | 1:6 |




Dynamics of circulating antibody during active immunization and after transfer of spleen cells from immune donors into adult nonirradiated recipients. 1) Recipients of living cells; 2) recipients of heated cells; 3) donors.

The hypothesis on the formation of antibody as a result of active immunization of the recipients also is refuted by the experimental data, inasmuch as during active immunization by Vi antigen antibody is not detectable during the first 48 h.

The data presented allow the conclusion that Vi antibody appearing in the recipient on the second day after transplantation is produced by the transplanted cells. Obviously under the experimental conditions used the transplanted cells continued intense antibody formation. This effect was constant and reproducible in all experiments.

During the next step of the study we investigated the functional dynamics of the antibody forming cells in situ both in the donor organism and following transplantation in the recipient organism. In experiments designed for this purpose animals were divided into three groups. The first group was immunized with Vi antigen (1 gamma intravenously). Cells from an "immune" spleen which had been obtained from donors 3 days after immunization were transferred into the animals of the second group. Mice in the third control group received the same cell suspension but it was previously heated to 56° for 30 min. Animals of all three groups were sacrificed at varying intervals – from 12 h to 10 days and Vi antibody was determined in their sera. The necessity of isolating the control group stems from the fact that antibody formation in the recipients at intervals after the time of transfer (begining at three days) can be conditioned not only by the activity of the transplanted cells but by the active reaction of the recipient cells.

These experiments were repeated 4 times and gave similar results. A summary of the data is given in the figure. They show the following. 1. Immunization of the donor mice by a preparation of Vi antigen in a dose of 1 gamma results in the appearance of Vi antibody in the serum on the third day. By the sixth day the antibody titer reaches a maximum after which it slowly declines. 2. Upon transfer of spleen cells from the immune donors to the nonimmune recipient specific antibody can be detected in the serum of the latter 12 h after the transfer. The titer of antibody reaches a maximum on the third day and thereafter declines. By the tenth day no antibody

is detectable in the recipient. 3. Upon transfer of a heated suspension of "immune" cells no Vi antibody is found in the recipients.

Special experiments carried out on 30 mice corroborated the well known thermal stability of Vi antigen and showed that heating the preparation does not lower its antigenic activity in mice. The injection of 1 gamma of Vi antigen heated together with spleen cells at 56° for 30 min evoked by the 5th day the same titer of Vi antibody (1:1280) as did the injection of 1 gamma of Vi antigen from an unheated preparation in saline. Consequently the absence of a humoral reaction in the recipient which received heated cells from an immune donor results from the fact that the material which was transferred contained no Vi antigen.

Thus antibodies which were formed in the recipients after transfer of living cells at all intervals following transfer could be accounted for by the activity of the transferred cells.

In analyzing the curves of antibody formation after transfer of "immune" cells two phases can be discerned. The first phase is characterized by a rapid rise in antibody titer because the amount of antibody provided by the transferred cells after the first 24 h on their residence in the recipient is some tenfold greater than the amount of antibody which is measured in the transferred material by titration of the extract. This rapid rise in antibody titer continues for 2 days after which the phase of sharp decline in titer ensues and continues directly to complete disappearance of antibody on the tenth day.

It is problematic whether in the second phase synthesis of antibody occurs or whether this portion of the curve reflects the process of natural degradation of immunoglobulin catabolized in the first three days of cellular function. To solve this problem an experiment was performed in which the rate of disappearance of antibodies occurred under conditions of passive immunization (the rate of natural degradation). It turned out that the rate of fall of titer under such conditions, when antibody was not being formed, approached the rate of disappearance of antibody in the experiments on transplantation (three to ten days after transfer of cells). These data indicate that transplanted cells form antibodies for three days after which begins a period of gradual destruction of the cells and disappearance of previously formed antibodies.

It is interesting to note that the maximal titer of Vi antibody upon transfer of immune spleen cells is observed after the same interval as is found for active immunization of animals – at the 5th to 6th day after contact with the antigen. The differences between the donor and the recipient is that in the former circulating antibody is found for a long time (more than 30 days) whereas in the latter they can only be detected for a short time.

The experimental model described by these experiments has the following distinctive features. 1. The transplantation of spleen cells in all experiments occurred successfully; the experimental method allows great reproducibility. 2. The transplanted cells from "immune" spleen produce antibody in high titer so that statistical verification is facilitated. 3. A single immunization of the donor is all that is necessary to achieve the desired effect from the cell transfer. 4. The transferred cellular material contains no antigen in an amount adequate to evoke antibody formation in the normal adult animal.

These features of the experimental model apparently result from the specific chemical preparation of Vi antigen used for immunizing the donor mice, evoking in them a very clear-cut humoral response. The proposed model is very convenient for the experimental study of immunologic function in lymphoid tissue.

## SUMMARY

Homotransplantation of splenic cells from mice immunized with a chemical Vi antigen preparation leads to an intensive formation of antibodies in the organism of normal adult recipients. No significant quantities of Vi antigen capable of provoking an active recipient reaction were present. The model permits study of the dynamics of the function of transplanted cells.

## LITERATURE CITED

1. D. D. Efimov, Zh. mikrobiol., No. 11 (1961), p. 115.
2. D. R. Kaulen, "Summaries of the papers of the 3rd inter-institute conference on radiation immunology and microbiology" (Moscow, 1962), p. 40.
3. V. L. Troitskii, M. A. Tumanova, and V. A. Izvekova, "Summaries of the papers of the 3rd inter-institute conference on radiation immunology and microbiology" (Moscow, 1962), p. 39.

4. Ya. Šterzl, Uspekhi covr. biol., No. 3 (1959), p. 356.
5. F. J. Dixon and W. O. Weigle, J. exp. Med., Vol. 110 (1959), p. 139.
6. T. Harris and S. Harris, in the book: Ciba Foundation Symposium on Cellular Aspects of Immunity (London, 1960), p. 172.
7. A. B. Stavitsky, J. Immunol., Vol. 79 (1957), p. 187.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.

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