# LYMPHOCYTE RESPONSE IN THE EARLY PERIOD AFTER HEART TRANSPLANTATION

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UDC 616.12-089.843-092.9-07:616.155.32-07

The quantitative and qualitative composition of the leukocytes and dehydrogenase activity of the lymphocytes were determined in blood entering and leaving the transplanted heart at different times after grafting in dogs. A decrease in the lymphocyte count in blood flowing from the grafted heart was found, on account of retention of the lymphocytes in the graft; the activities of the mitochondrial dehydrogenases also were reversed as the result of contact of the lymphocytes with the foreign antigens of the transplanted heart. It is suggested that the mechanism of reversal is connected with the functions of the lymphocytes as immunocompetent cells.

KEY WORDS: heart transplantation; lymphocytes; dehydrogenases.

Despite an extensive literature on the dynamics of development of transplantation immunity and the mechanisms responsible for manifestation of immunologic conflict, the processes taking place in the early post-transplantation period have not yet been adequately studied. In particular, the problem of changes in the lymphocytes following their primary contact with the foreign antigens of the graft is not clear. Yet the solution of this problem would widen our ideas of the mechanisms responsible for the development of the productive phase of immunity.

The object of this investigation was to study the dynamics of quantitative and qualitative changes in the white blood cells as a result of their contact with the grafted heart during the first few hours after transplantation. For this purpose a comparative morphological investigation was made of blood entering and leaving the graft, during which special attention was paid to the cytochemical determination of activity of the mitochondrial dehydrogenases of the lymphocytes, namely succinate (SD) and $\alpha$-glycerophosphate ($\alpha$-GPD) dehydrogenases.

Data in the literature [2, 3, 9] are evidence that changes take place in the enzyme activity of the lymphocytes during immunogenesis and, in particular, during the development of transplantation immunity [1, 5]. The choice of mitochondrial dehydrogenases for study was dictated by the results of the writers' previous investigations [12], in which reversal of the activities of $\alpha$-GPD and SD was found to take place in the late stages of immunologic conflict after heart transplantation. Later, a similar phenomenon was discovered in experimental and clinical investigations after transplantation of skin [4] and the kidneys [7].

## EXPERIMENTAL METHOD

Experiments were carried out on mongrel dogs. The recipients weighed 20-22 kg, the donors 12-15 kg. Transplantation of the heart was carried out in two modifications: on the iliac vessels [10] in three experiments and on the cervical vessels [14] in five experiments. Blood entering the graft was obtained for investigation from the cannula connecting the carotid artery and subclavian artery of the donor's heart. Blood leaving the graft was obtained from the cannula connecting one branch of the pulmonary artery of the donor's heart with the recipient's external jugular vein. Blood was taken before the beginning of transplantation (background) and 30 min and 2 and 4 h after transplantation. In five control experiments, on the dog's own heart, the effect of anesthesia (hexobarbital) and hypoxia on the leukocyte count and metabolism of the lymphocytes was studied in blood entering and leaving the heart.

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 0007-4888/78/8506-0770$07.50 

TABLE 1. Composition of Leukocytes and Dehydrogenase Activity of Lymphocytes in Blood Entering (A) and Leaving (B) Recipient's Transplanted Heart (8 experiments)

| Index | Background | 30 min | | 2 h | | 4 h | |
|---|---|---|---|---|---|---|---|
| | | A | B | A | B | A | B |
| Leukocytes | 9258 (6700—12 150) | 10 232 (6500—14 400) | 9958 (5900—13 700) | 14 381 (8800—23 900) | 14 100 (8800—23 900) | 16 500 (11 700—22 400) | 14 466 (11 700—19 600) |
| Neutrophils | 4747 (1576—6700) | 6089 (2352—11 232) | 6512 (2343—11 645) | 11 559 (7200—16 360) | 11 168 (6400—19 600) | 13 916 (10 530—18 600) | 12 933 (10 180—17 250) |
| Lymphocytes | 1950 (1190—2875) | 2344 (1820—2829) | 1693 (1096—2345) | 1841 (840—3170) | 1788 (875—3350) | 1858 (1055—3136) | 720 (240—1568) |
| | | $P<0,05$ | | | | $P<0,05$ | |
| Monocytes | 452 (122—492) | 466 (120—720) | 531 (118—940) | 690 (400—1010) | 634 (350—1045) | 343 (120—460) | 408 (363—470) |
| Eosinophils | 2109 (164—4488) | 1333 (288—3636) | 1212 (59—3264) | 291 (180—840) | 510 (88—2037) | 383 (224—924) | 405 (121—702) |
| SD | 22,5 (29,2—18,0) | 21,4 (19,7—24,6) | 20,5 (18,8—23,3) | 19,9 (17,4—22,7) | 19,3 (17,2—21,2) | 21,1 (18,2—24,6) | 21,3 (20,2—22,4) |
| α-GPD | 19,8 (16,9—22,0) | 19,4 (15,1—21,5) | 24,5 (19,4—43,8) | 18,5 (17,1—21,1) | 20,7 (18,6—25,2) | 19,3 (17,4—20,4) | 21,6 (20,1—23,6) |
| SD/α-GPD | 1,12 (1,03—1,14) | 1,11 (1,01—1,32) | 0,89 (0,49—1,03) | 1,07 (1,01—1,16) | 0,94 (0,81—1,02) | 1,09 (1,01—1,23) | 0,99 (0,91—1,06) |
| | | $P<0,05$ | | $P<0,05$ | | $P<0,05$ | |

Note. Limits of variations shown here and in Table 2 in parentheses.

TABLE 2. Composition of Leukocytes and Dehydrogenase Activity in Lymphocytes from Blood Entering (A) and Leaving (B) Recipient's Own Heart (five experiments)

| Index | Background | Background 30 min later | | Hypoxia for 30 min | | Hypoxia for 2 h | |
|---|---|---|---|---|---|---|---|
| | | A | B | A | B | A | B |
| Leukocytes | 6642 (5250—8500) | 6089 (3750—7750) | 6344 (4800—7150) | 6785 (4000—8750) | 6790 (5250—7950) | 6290 (3500—9750) | 5180 (4000—7550) |
| Neutrophils | 5247 (4245—6889) | 4993 (3400—5776) | 5075 (4368—5863) | 5564 (3480—7565) | 5704 (4830—6678) | 5032 (2835—7215) | 4196 (3160—5436) |
| Lymphocytes | 863 (53—1048) | 670 (75—1155) | 761 (240—1474) | 651 (146—1750) | 679 (105—2015) | 755 (184—1658) | 673 (152—1510) |
| Monocytes | 532 (210—765) | 426 (113—822) | 508 (190—840) | 570 (160—1173) | 407 (280—636) | 503 (105—1073) | 311 (120—604) |
| SD | 24,6 (21,0—28,2) | 26,3 (22,4—30,7) | 23,5 (20,3—26,8) | 26,1 (21,8—29,4) | 23,9 (21,0—27,6) | 25,6 (22,2—28,8) | 24,7 (21,7—27,0) |
| α-GPD | 20,2 (18,0—23,0) | 20,5 (17,8—24,4) | 19,2 (18,5—20,5) | 18,8 (13,3—22,9) | 19,5 (18,8—20,8) | 20,6 (18,4—23,0) | 19,6 (18,2—20,8) |
| SD/α-GPD | 1,22 (1,08—1,48) | 1,28 (1,26—1,32) | 1,22 (1,10—1,42) | 1,39 (1,15—1,64) | 1,22 (1,13—1,47) | 1,24 (1,16—1,35) | 1,26 (1,16—1,39) |

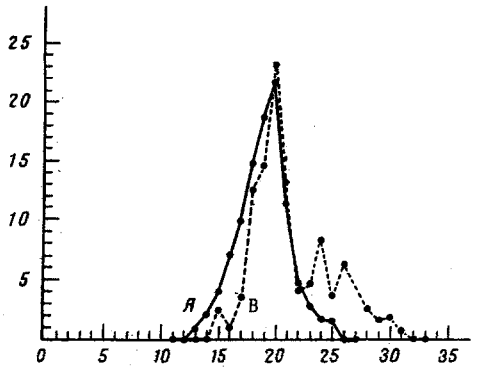


Fig. 1. Changes in α-GPD activity in blood entering (A) and leaving (B) recipient's transplanted heart 2-4 h after grafting. Abscissa, number of granules per cell; ordinate, percentage of lymphocytes.

The number and qualitative composition of the leukocytes were determined by the usual methods and the dehydrogenase activity of the lymphocytes was studied by Nartsissov's method [8], based on counting the number of formazan granules formed by interaction between the lymphocytes and appropriate substrates in the cell. The results were subjected to statistical analysis by Student's method.

## EXPERIMENTAL RESULT

In all the experiments carried out on different models of heterotopic heart transplantation consistent results were obtained, so that they could be considered together. As Table 1 shows, 2 h after transplantation, a significant increase in the number of leukocytes was observed in the recipient's blood entering the graft, which continued until 4 h after the operation. It was due to an increase in the number of neutrophils ($P < 0.05$) at all periods of the investigation, and was evidently the result of the development of inflammation in the wound. No significant changes took place in the numbers of the other blood cells at these times, except for an increase in the number of monocytes 2 h after transplantation ($P < 0.05$), further confirmation of the inflammatory nature of this leukocytosis.

Comparison of the composition of the leukocytes in blood entering and leaving the graft gave the following results: the number of neutrophils was unchanged during passage of the blood through the graft. The small decrease found in blood flowing from the graft after 4 h was not statistically significant. Monocytes also were not held up in the graft. Meanwhile, the number of lymphocytes in the outflowing blood was significantly smaller as early as 30 min after transplantation ($P < 0.05$). This difference could no longer be found after 2 h, but after 4 h it was again clearly defined. It can thus be postulated that the retention of the recipient's lymphocytes in the graft was observed after the first few minutes of their contact with the foreign tissue.

Changes in mitochondrial dehydrogenase activity in the lymphocytes after their contact with the graph also was noted. As Table 1 shows, the activity of these enzymes in the general circulation (A) showed no significant changes during the experiment, although a tendency for their activity to decrease could be detected. Comparison of the SD level in cells from blood entering the graft and after their passage through the coronary vessels of the transplanted heart (B) showed no significant change in activity of the enzymes. Under these conditions the α-GPD activity of the lymphocytes regularly increased. Whereas in the inflowing blood the ratio between the activities of the enzymes (SD > α-GPD) was that ordinarily found in lymphocytes [12], after their contact with the tissues of the graft there was a significant reversal of the activities (SD < α-GPD) of the enzyme studied at all periods of the investigation. During analysis of the distribution of the activities of the enzymes in the lymphocyte population it was found that the increase in α-GPD activity perhaps depends on the appearance of a subpopulation of activated cells with a high content of the enzyme (Fig. 1).

The observed changes in dehydrogenase activity of the lymphocytes in the early stages after transplantation of the heart could depend on several causes. They could be: 1) a consequence of the trophic function of the lymphocytes and correlation of their metabolism with that of the myocardium [9], i.e., a manifestation of the ordinary change taking place in lymphocytes through the coronary system; 2) a reflection of hypoxia of the graft, taking place during transplantation when the heart was transferred from donor to recipient [14]; 3) the

result of the response of the lymphocytes to foreign antigens of the graft. This last hypothesis was concerned by previous investigations [11, 13], when the writers discovered that identical changes in metabolism develop in lymphocytes during the rejction reaction. To test these hypotheses control experiments were carried out in which dogs were anesthetized with hexobarbital, thoracotomy performed, and the heart cannulated: the aorta (inflowing blood) and the coronary sinus (blood flowing from the myocardium). After the quantitative and qualitative composition of the leukocytes had been determined twice (at intervals of 30 min) in the inflowing and outflowing blood, general hypoxia was produced in the dogs by reducing the ventilation of their lungs, and the same blood indices were again determined. The results of these experiments (Table 2) showed no significant changes either in the numbers of the leukocytes or in the activity of their mitochondrial dehydrogenases. The $\alpha$-GPD and SD activity in blood entering and leaving the heart were the same and the ratio between their activities was always normal, i.e., more than 1.

Consequently, operative trauma, the trophic function of the lymphocytes, and the state of hypoxia were not the causes of the change in the ratio between dehydrogenase activities discovered in the main experiments. The correct explanation, it seems, is evidently that reversal of the activities of SD and $\alpha$-GPD is the result of contact between the lymphocytes and the antigens of the graft, i.e., that its mechanism is identical with that observed at the beginning of development of the rejection crisis, and is evidently connected with the functions of lymphocytes as immunocompetent cells. However, if the response of the lymphocytes in the early stages after transplantation and in the terminal period of function of the heart is compared, a significant difference will be noted. During the first few hours after transplantation the number of immunocompetent cells responding by reversal of dehydrogenase activities was so small that it could be detected only by investigating blood taken immediately after contact with the graft. The study of the peripheral blood or blood taken from the aorta did not reveal reversal, probably because of dilution of blood flowing from the graft in the general circulation.

A different picture was observed during reversal of the dehydrogenase activities of the lymphocytes in the late period after transplantation. In these cases a reversed ratio between the dehydrogenase activities (SD < $\alpha$-GPD) was observed in most lymphocytes, and because of this the reversal could be detected in peripheral blood films also.

It can thus be tentatively suggested that at the beginning of formation of the immunologic response to the graft, just as at the beginning of immunologic conflict, characteristic changes take place in the mitochondrial dehydrogenases of the antigen-reactive lymphocytes, based on the need for rapid mobilization of the energy resources of the cell [6].

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