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Studies on the reaction of host lymphoid system in homotransplantation. I. Humoral and cellular reactions against the transplanted cells

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Studies on the reaction of host lymphoid system in homotransplantation. I. Humoral and cellular reactions against the transplanted cells*

Yoshiaki Kokumai

Abstract

For the purpose to clarify the relationship between production of humoral antibodies and cellular reactions of the lymphoid system to allogeneic-transplanted cells in mice, a study on cross sensitization was carried out between inbred A(H-2a) and C3H(H-2k) strain mice. The median survival time of skin of C3H transplanted to A (C3H-to-A) was 14.1 ± 1.4 days, and of A transplanted to C3H(A-to-C3H) was 11.8 ± 1.6 days. Repeated A cell injections to C3H induced the formation of humoral antibodies, whereas the C3H cell injections into A did not. In A-to-C3H and C3H-to-A combinations, the immunization induced an increase in white blood cell number in circulating blood successively with the repetition of the antigen injection, and organ weights increased in thymus, spleen, and liver but not in kidney. Weight increases in the organs of A treated with C3H cell injection were less in extent, comparing to those of C3H treated with A cells. Histologic observations revealed that the cellular proliferation in the lymphoid system including plasmocytic responses were obviously predominant in the C3H treated with A cells comparing to those in the A treated with C3H cells. Hemocytoblasts also increased during the immunization in both cases showing no significant differences between the two series of experiments. These cellular reactions were observed not only in the draining lymph nodes but also in the generalized lymphoid tissues. The results of the present study suggest that the definitive factor for producing humoral antibodies is in the differences of the homologous antigenicity between the donor and the recipient but not in the degree of sensitization, and the Dk in H-2 loci is not so strong in antigenicity as to elicit sufficient plasmocytic responses for the formation of humoral antibodies in C3H strain mouse.

**STUDIES ON THE REACTION OF HOST LYMPHOID
SYSTEM IN HOMOTRANSPLANTATION**

**I. HUMORAL AND CELLULAR REACTIONS AGAINST
THE TRANSPLANTED CELLS***

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The evidence that homograft rejection is a manifestation of immunity has been unequivocal¹, and also it has been verified that the cells of lymphocyte series play an active role in these rejection phenomena^{2,3}. By the many evidences that the transplantation immunity can be transferred passively by lymphoid cells from preimmunized host⁴⁻⁶ but not by serum⁷⁻¹⁰, immunologic mechanisms involved in homograft rejection and tuberculin allergy were suggested to be similar modalities of delayed-type hypersensitivity. The immune serum shows, however, strongly cytotoxic activities in certain systems, both *in vitro* and *in vivo*¹⁶⁻¹⁸, and it has been gradually recognized that immune serum can bring about homograft rejection¹¹⁻¹⁵. As a matter of fact, the relative role played by these two components, i. e. cell-bound antibodies and humoral antibodies in graft rejection, is a matter of controversy at the present time. On the other hand, it has been demonstrated that humoral antibodies are elaborated by the cells of plasma cell series¹⁶⁻²² and immature lymphocytes²³⁻²⁵. Now, there arises a problem of an utmost importance as to whether there is substantial difference between humoral antibodies and cell-bound antibodies²⁶⁻²⁸. Under these circumstances the cells qualified to undertake immunological responses are summarized in the symbolical term of immunologically competent cells²⁹.

In homotransplantation, some systematic long-term studies of the lymphoid responses, especially the reactions of immunologically competent cells^{30,31}, and the results of long-term observation of the humoral antibodies³² have been reported separately in detail, but there is no quantitative study simultaneously examining these two components.

As an approach to dynamics of transplantation immunity, the present study was designed to examine the relationship between cellular reactions of the

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lymphoid system and formation of humoral antibodies to homotransplanted cells in mice considering histocompatibility differences.

MATERIALS AND METHODS

Animals : The following inbred strains of male adult mice from the Research Colonies of the Okayama University Medical School were used : A (H-2^k) and C3H (H-2^k).

Transplantation antigens : Living spleen and lymph-node cells were used. The spleens and lymph nodes collected, with all required aseptic precautions, were gently chopped and expressed through a standard screen sieve (opening 0.149mm, U. S. No. 100) into buffered normal saline. The cells isolated by vigorous pipetting were counted, disregarding the red cells. The suspensions were used with a cell concentration of 200 million per ml.

Sensitization : Cross sensitization between A strain and C3H strain mice was made. The antigenic material was injected by the intraperitoneal route 4 times every 7 days. Dosage of cells per injection per mouse was 20×10^6 , 40×10^6 , 160×10^6 and 160×10^6 respectively.

Skin grafting : Skin grafting was done by the modified method of BILLINGHAM and MEDAWAR³³. The numbers of surviving grafts were recorded from day to day, and the results were summarized in the form of a median survival time. All survival times are defined in terms of endpoints, not in terms of the time at which breakdown began.

Hemagglutination test : Pooled antisera from three mice were titrated by the hemagglutination technique, with slight modifications, in the presence of dextran and absorbed human serum³⁴. The tubes used for this test were 8mm in diameter and 70mm long. Reagents were mixed in the ratio of 0.05ml antiserum in doubling dilution in 1.8 per cent dextran to 0.05ml of a 2 per cent suspension of donor erythrocytes in 50 per cent v/v absorbed human serum. The tubes were incubated for 90 minutes at 37°C and centrifuged at 600g for 30 seconds. The degree of agglutination was observed macroscopically by gentle agitation, and was verified microscopically, particularly to establish endpoints. Two control tubes were regularly used : in the first, antiserum was replaced by 1.8 per cent dextran ; in the second, absorbed human serum was added. The specificity of the reaction was tested with isologous red cells. The readings were graded as follows : complete agglutination (no free cells), strong and weak agglutination, doubtful, and no agglutination. Only the first three groups were regarded as positive.

Cytotoxic test : According to the method of GORER and O'GORMAN¹⁶, with slight modifications, 0.05 ml of a suspension containing 10^7 donor lymphoid

cells per ml was added to 0.05 ml antiserum diluted serially in Ringer's solution, finally, 0.05 ml guinea-pig serum was added to each tube and the tubes were incubated for 90 minutes at 37°C. After incubation the approximate proportion of viable cells was estimated by the eosin method of SCHREK³⁶. About 300 cells were rapidly counted in a hemocytometer and classified as stained or unstained. In the controls antiserum was replaced by Ringer's solution. For each antiserum dilution a cytotoxic index was calculated according to HELLSRTRÖM³⁶, defined as the difference between the proportion of unstained cells in the control and the test suspension, divided by the former figure. The endpoint corresponds to the highest dilution which gave a cytotoxic index ≤ 0.15 .

Organ-enlargement assays: The weight-gain assays, first devised by SIMONSEN and co-workers³⁷ were applied to the thymus, spleen, liver and kidneys. The formula of organ index is given by relative organ weight of experimental mouse divided by relative organ weight of control mouse. The relative organ weight is the ratio of the organ weight to the body weight of each mouse.

Processing of tissue: The smears of draining lymph nodes (mesenteric), distant lymph nodes (cervical), and spleens were stained with May-Giemsa and methyl green pyronin. A differential count of 1,000 cells was made from these smears of each animal. The nomenclature of the various cell types followed the recommendations proposed at the Prague Meeting on Mechanism of Antibody Formation³⁸. This nomenclature includes the use of the term hemocytoblast for the large, probably immunologically competent, cell which appears during immune proliferative reaction.

In each experimental group, three mice were sacrificed and tested every two or three days until the fourth week after the first injection of the homologous lymphoid cells, and after that time every 7 or 10 days until the end of the sixth week.

RESULTS

1. Survival time of skin homografts exchanged between A strain and C3H strain mice

A life table recording the mortality day by day of male A strain backskin grafts on 10 normal C3H males and vice versa is given in Table 1. As shown in Figure 1, the rejection curves of these two groups parallel considerably, and the difference is statistically significant at 95 per cent confidence limits. In the C3H-to-A strain combination the median survival time (MST) is 14.1 days, with a standard sampling error of 1.4 days, and no graft survived longer than 18 days. In the reverse A-to-C3H strain combination the MST is 11.8 ± 1.6 days, and no graft survived longer than 16 days.

Table 1 Survival of Skin Homografts Exchanged Between A and C3H
The figures show the number of surviving grafts as observed daily after transplantation.

Exp.	Days After Transplantation																					
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1. C3H→A	10	10	10	10	10	10	10	10	10	10	10	8	7	6	5	2	2	1	0	0	0	0
2. A→C3H	10	10	10	10	10	10	10	10	9	9	5	5	4	3	2	1	0	0	0	0	0	0

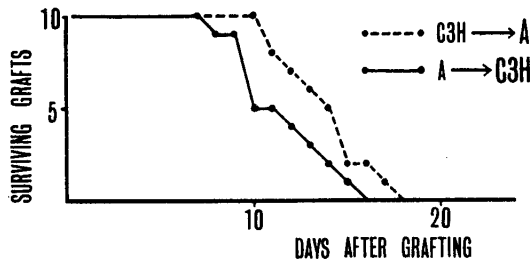


Fig. 1 Rejection curves of skin homografts exchanged between A and C3H

2. Cytotoxic and hemagglutinating antibody titers

Cytotoxic and hemagglutinating antibody titers of pooled sera obtained at intervals during immunization are shown in Figure 2. In the C3H-to-A strain combination these humoral antibody titers were followed up until the third week after the fourth sensitization, but humoral antibodies were not at all detected. On the other hand, in the reverse A-to-C3H strain combination hemagglutinin appeared at the seventh day after the first sensitization and cytotoxin appeared after

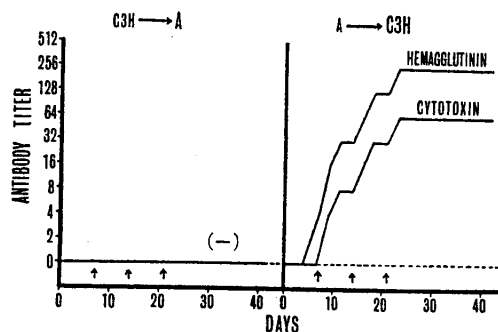


Fig. 2 Cytotoxic and hemagglutinating antibody titers of pooled sera obtained at intervals during the immunization with homologous lymphoid cells. Initial injection on day 0 was 20×10^6 cells, and the mice were boosted stepwise as indicated by the arrows, 40×10^6 , 160×10^6 , and 160×10^6 cells. All the injections were made through i. p. route.

the second sensitization. Thereafter both titers were increased by repeated sensitizations, and after the fourth sensitization they were elevated simultaneously to the highest level. These titers were 256 in hemagglutinin and 64 in cytotoxin.

3. White blood corpuscles (WBC) in the peripheral blood

Variations of WBC in the peripheral blood are shown in Figure 3. In both

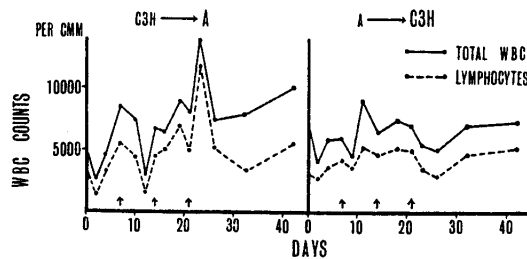


Fig. 3 White blood corpuscles in the peripheral blood. The arrows and day 0 indicate the time of the sensitization.

combinations the number of WBC was increased after repeated sensitizations with a slight diminution just afterwards. The values varied considerably in the C3H-to-A strain combination rather than in the other. As indicated in Figure 3, variations of the total WBC show a fairly parallel relationship with those of lymphocytes in the peripheral blood.

4. Organ-enlargement assays

Organ-enlargement assays were carried out for the thymus, the spleen, the liver and the kidneys. To begin with weight-gain assays of these organs in the sensitized adult mice, a preliminary experiment was performed as to determine individual differences of relative organ weight (organ weight/body weight) in normal adult mice. The results are illustrated in Table 2. Except for the thymus, the individual differences of the relative organ weight were minimal to the extent of insignificance. There were individual variations in the relative thymus weight to some degree, probably because of involutory organ in the adult.

Organ indices of the thymus, the spleen, the liver and the kidneys after sensitization are shown in Figure 4. Because the mice used in this study are adult, of which the body weight is almost constant throughout the experiments, it seems probable that an increase in the organ index may reflect the rate of organ enlargement. In the C3H-to-A strain combination without formation of humoral antibodies, the thymus and spleen indices increased beyond 1, but there were not any remarkable variations in the liver and kidney indices. In the A-to-C3H strain combination with formation of humoral antibodies, the spleen index resulted in a marked increase and at the fourth day after the fourth sensitization

Table 2 Relative Organ Weights (organ weight/body weight) of Normal Mice

Strain	Mouse No.	Body Weight (g)	Relative Spleen Weight ($\times 10^{-5}$)	Relative Liver Weight ($\times 10^{-4}$)	Relative Thymus Weight ($\times 10^{-6}$)	Relative Kidney Weight ($\times 10^{-4}$)
A	1	33.5	358	642	896	137
	2	30.5	367	479	721	151
	3	31.5	362	492	222	181
	4	33.4	347	527	1200	141
	5	34.6	358	552	954	165
	6	31.0	365	465	871	145
	7	27.5	364	436	1018	135
	8	26.6	387	432	865	143
	9	29.1	392	430	997	141
	10	24.7	385	433	851	178
Rel. Organ WT. \pm S. E.			369 \pm 9	489 \pm 12	870 \pm 152	152 \pm 10
C3H	1	21.5	412	451	186	158
	2	18.9	360	508	582	153
	3	17.7	367	441	452	158
	4	19.5	359	451	410	164
	5	18.7	406	508	802	144
Rel. Organ WT. \pm S. E.			381 \pm 16	472 \pm 32	486 \pm 182	155 \pm 6

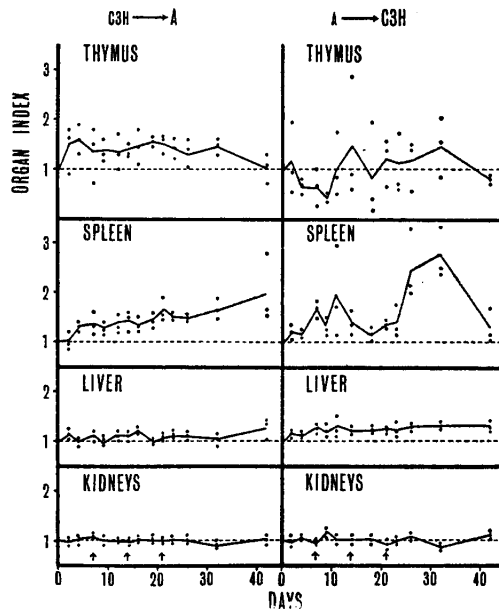


Fig. 4 Organ-enlargement assays. Organ indices of the thymus, spleen, liver and kidneys are those in the C3H-to-A and A-to-C3H strain combinations.; the base line of zero weight gain (Index=1). —; mean values of 3 mice.

it was increased to 2.78 in average value of three mice. The gross appearance of the enlarged spleen at that time is compared with that of normal mouse in Photograph 1. The liver index was also increased slowly, but there was no significant variation in the kidney index. The thymus showed temporary, negative weight gain after the first sensitization.



Photo 1 Enlarged spleens in the A-to-C3H strain combination on the fourth day after the fourth sensitization. A) The spleen of normal mouse (spleen weight 0.070 g/body weight 19.5 g). B) The spleens of sensitized mice (0.185 g/19.6 g, 0.268 g/21.0 g, 0.160 g/17.3 g respectively).

5. Cellular reactions of the lymphoid system

Tables 3 and 4 summarize the results obtained as to cellular reactions of the

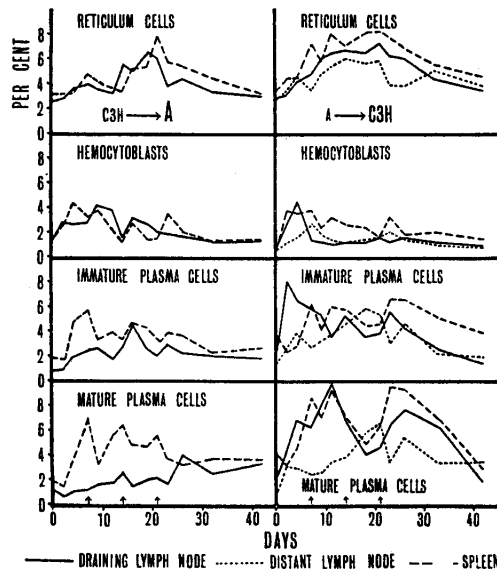


Fig. 5 Variations of the immunologically competent cells of the lymphoid tissues in the C3H-to-A and A-to-C3H strain combinations during homotransplantation reactions.

Table 3 Cellular Constituents of the Lymphoid Tissues in the C3H-to-A Strain Combination During Homotransplantation Reactions
The figures show per cent of the cells in 1,000 differential counts.

Day	Hemocytoblasts		Immature Plasma Cells		Mature Plasma Cells		Eosinophils		Reticulum Cells	
	Draining Node	Spleen	Draining Node	Spleen	Draining Node	Spleen	Draining Node	Spleen	Draining Node	Spleen
0	1.4±0.3	1.4±0.5	1.0±0.2	1.9±0.4	1.2±0.2	1.8±1.2	0.3±0.1	0.3±0.1	2.7±0.7	3.2±2.9
2	2.8±0.7	2.6±0.2	1.0±0.5	1.8±0.5	0.7±0.2	1.4±0.1	0.2±0.1	0.2±0.1	2.8±0.7	3.2±0.8
4	2.7±0.6	4.5±0.5	1.8±0.5	4.7±1.5	1.1±0.4	3.5±1.0	0.2±0.2	0.3±0.2	3.7±1.1	3.3±1.7
7	2.8±0.6	3.2±1.0	2.3±1.0	5.8±1.7	1.3±0.7	7.0±2.9	0.2±0.1	0.3±0.2	4.0±2.2	4.9±1.6
9	4.1±1.5	3.9±1.0	2.6±1.0	3.2±0.3	1.7±1.1	3.2±1.5	0.4±0.1	0.4±0.2	3.5±1.0	4.2±1.2
12	3.8±1.7	2.2±0.2	1.9±0.7	3.8±0.3	1.9±0.5	5.6±0.6	0.2±0.1	0.2±0.2	3.2±1.8	3.8±1.7
14	1.6±0.7	1.3±0.4	2.9±1.0	3.2±0.3	2.7±0.7	6.5±1.2	0.6±0.1	0.8±0.4	5.7±1.4	3.5±0.4
16	3.1±1.2	2.8±1.0	4.6±2.0	4.7±1.4	1.5±0.4	4.8±1.5	0.3±0.1	0.4±0.2	5.0±1.0	5.1±2.3
19	2.5±0.8	1.4±0.2	2.7±1.0	4.2±1.1	2.0±0.2	4.7±1.2	0.2±0.1	0.6±0.3	6.6±2.4	5.5±2.3
21	2.0±1.1	1.5±0.5	2.0±0.3	3.1±1.2	2.3±0.9	5.6±1.5	0.4±0.1	0.4±0.1	6.0±2.4	7.9±2.4
23	1.9±0.1	3.5±0.9	3.1±1.4	3.9±0.5	1.7±0.9	2.7±1.0	0.7±0.4	0.9±0.3	3.8±1.4	5.7±1.1
26	1.7±0.6	2.0±2.0	2.3±0.3	3.7±1.1	4.1±1.0	3.2±0.8	0.2±0.1	0.2±0.1	4.3±1.6	5.4±3.6
32	1.2±0.2	1.2±0.3	2.1±0.3	2.2±0.7	2.5±1.3	3.7±0.1	0.2±0.1	0.5±0.2	3.7±0.6	4.3±1.1
42	1.3±0.2	1.3±0.2	1.8±0.3	2.6±0.5	3.3±1.0	3.6±0.2	0.4±0.1	0.4±0.2	3.0±1.1	3.2±0.6

Table 4 Cellular Constituents of the Lymphoid Tissues in the A-to-C3H Strain Combination During Homotransplantation Reactions
The figures show per cent of the cells in 1,000 differential counts.

Day	Hemocytoblasts			Immature Plasma Cells			Mature Plasma Cells			Eosinophils			Reticulum Cells		
	Draining Node	Distant Node	Spleen	Draining Node	Distant Node	Spleen	Draining Node	Distant Node	Spleen	Draining Node	Distant Node	Spleen	Draining Node	Distant Node	Spleen
0	1.0±0.5	0.9±0.3	1.0±0.5	3.0±1.5	1.2±0.3	3.6±1.1	2.1±0.4	0.7±0.3	3.9±1.1	0.1±0.1	0.1±0.1	0.3±0.1	2.9±1.3	2.9±0.6	3.5±1.2
2	2.7±0.7	1.2±0.8	3.8±1.9	3.0±6.2	2.5±2.4	2.3±0.5	4.3±5.4	3.1±3.4	3.0±1.2	0.1±0.1	0.0±0.1	0.1±0.1	13.1±1.1	3.5±1.9	4.4±1.4
4	4.5±0.8	1.6±0.4	3.5±0.5	6.5±1.7	3.8±2.4	2.8±0.5	6.8±1.3	2.9±1.4	4.5±1.7	0.2±0.1	0.1±0.1	0.1±0.1	14.1±1.0	4.5±2.6	4.3±1.1
7	1.2±0.3	2.7±2.1	3.8±3.8	5.8±0.5	2.7±0.4	6.2±3.2	6.3±0.7	2.4±0.5	8.7±1.4	0.2±0.2	0.2±0.2	0.3±0.1	14.9±1.0	3.4±1.1	7.3±0.7
9	1.2±0.3	1.8±0.8	2.3±0.3	5.2±1.0	3.1±1.4	4.0±1.0	8.2±2.8	2.6±1.9	7.0±2.9	0.1±0.1	0.1±0.1	0.2±0.1	16.1±2.1	4.9±0.4	5.8±1.0
11	1.0±0.2	1.4±0.2	3.2±1.0	3.4±0.7	3.7±0.4	6.0±0.8	10.1±3.8	3.4±2.0	9.3±5.5	0.1±0.2	0.1±0.1	0.4±0.3	6.5±0.7	5.3±1.3	8.1±1.3
14	1.3±0.7	1.3±0.7	2.7±2.0	5.3±3.9	4.9±1.0	5.7±1.4	6.9±2.3	3.8±0.8	7.0±1.8	0.2±0.1	0.1±0.1	0.6±0.5	6.7±1.3	6.1±2.3	7.0±0.2
18	1.2±0.3	1.5±0.6	2.3±1.8	3.5±0.0	5.9±2.4	4.5±0.8	4.0±2.4	5.8±1.8	4.8±1.4	0.1±0.1	0.2±0.1	0.4±0.1	16.5±2.4	5.7±1.7	8.1±2.0
21	1.5±0.8	1.6±0.7	1.6±0.8	3.8±1.0	5.2±2.4	4.6±0.7	4.5±1.2	6.5±3.4	6.2±0.8	0.2±0.2	0.4±0.2	0.9±0.6	7.3±1.4	5.8±0.7	8.1±2.0
23	1.2±0.2	2.0±0.9	3.2±0.7	5.6±0.6	2.9±0.4	6.5±2.9	6.3±2.2	3.1±2.4	9.5±2.5	0.1±0.1	0.2±0.3	0.5±0.5	6.3±0.4	3.9±1.0	5.7±1.6
26	1.5±0.5	1.2±0.9	1.8±0.3	4.2±1.4	4.7±1.6	6.5±1.3	7.6±2.0	5.4±0.3	9.2±2.1	0.0±0.1	0.1±0.1	0.3±0.2	26.0±1.9	3.8±1.3	6.9±2.6
32	1.1±0.3	1.0±0.5	2.0±0.5	2.3±1.2	2.1±0.7	5.0±0.8	6.1±2.6	3.3±0.4	6.8±1.8	0.2±0.2	0.2±0.2	0.5±0.3	4.5±1.0	5.1±0.5	5.7±1.6
42	0.9±0.1	0.8±0.3	1.5±0.3	1.3±0.6	1.9±0.9	2.8±0.4	1.8±0.6	3.5±0.9	2.9±0.4	0.1±0.1	0.2±0.2	0.3±0.1	13.5±0.7	3.8±0.4	4.9±1.0

Reaction of Lymphoid System in Homotransplantation

lymphoid tissues during immunization with allogeneic lymphoid cells. Table 3 shows the results in the C3H-to-A strain combination without formation of humoral antibodies, and Table 4 illustrates the results in the A-to-C3H strain combination with formation of humoral antibodies. Figure 5 also indicates the variation curves of these immunologically competent cells during immunization except for eosinophils.

Generally speaking, these observations reveal that the cellular reactions to the homotransplanted cells are the systemic ones. As seen in these results, there were marked changes of cellular constituents in the smears of the draining lymph node as well as in the smears of the spleen and the distant lymph node. These changes were represented by increasing numbers of reticulum cells, hemocytoblasts, immature plasma cells, and mature plasma cells with the lapse of time after the first sensitization. A far higher absolute increase of these cells may be inferred from the marked hypertrophy of the organs as indicated by organ-enlargement assays.

The characteristic cell in the lymphoid response to homografts is the hemocytoblast. A marked proliferation of hemocytoblasts was likewise seen in this experimental system. The number of hemocytoblasts in the smears of the draining lymph node of C3H recipient reached the peak by the fourth day after the first sensitization (4.5 per cent), and so in the smears of the draining lymph node of A recipient by the second day after the second sensitization (4.1 per cent).

The outstanding proliferation of plasma cells was seen in the C3H recipient producing humoral antibodies. In the draining lymph node the number of immature plasma cells reached the peak by the second day after the first sensitization (8.0 per cent), and so the number of mature plasma cells by the fifth day after the second sensitization (10.1 per cent). In the spleen the plasmocytic reactions began several days later than in the draining lymph node, and in the distant lymph node the reactions developed more slowly and mildly. On the other hand, in the A recipient not producing humoral antibodies the plasmocytic reactions were not so conspicuous as in the C3H recipient and the reactions were rather marked in the spleen than in the draining lymph node.

DISCUSSION

All the experimental works performed in recent years indicate that transplantation of living tissues or injection of living cells elicits both transplantation immunity and humoral immunity³⁹. The humoral immunity can be demonstrated by the appearance of isoantibodies, especially by hemagglutinins^{34,40}. Many other humoral antibodies such as hemolysins⁴¹, leuco-agglutinins⁴², and cytotoxins³⁹

may be identified, but the hemagglutinin reaction devised by GORER⁴⁴ employing dextran and absorbed human serum is perhaps the easiest and the most accurate one. Mouse anti-mouse-H-2 sera do not give precipitin lines with H-2 antigen preparations in agar presumably because H-2 antibody is incomplete⁴⁵.

A and C₃H strain mice used in this experiment differ in H-2 histocompatibility system respectively, the former belongs to H-2^a and the latter belongs to H-2^k⁴⁶. Theoretically, because of these differences in H-2 antigenicity, the C3H-to-A strain combination should give anti-D^k antibodies, and the A-to-C3H strain combination anti-FJMN antibodies, so that these humoral antibodies should have been detected by hemagglutination test and/or cytotoxic test. Unexpectedly, however, the C3H-to-A strain combination did not give any detectable humoral antibodies even under hyperimmunization so far as this experiment was concerned. HILDEMAN and MEDAWAR⁴⁷ recently pointed out that if the combination A donor and CBA recipient is indeed serologically most favorable, the reverse combination CBA donor and A recipient is much less favorable and does not give regularly detectable hemagglutinins. Since C3H and CBA strain mice belong to the same H-2^k group, their proposition coincides with our finding. In the present study consequently, systemic manifestations of the lymphoid system during homotransplantation reactions in the A-to-C3H strain combination producing humoral antibodies were compared with those in the C3H-to-A strain combination not producing humoral antibodies.

The majority of classic studies concerning the relationships of the lymphoid system to the homograft destruction revealed that the lymphoid system has a decisive function in transplantation immunity. In a classic study of skin grafts in rabbits, MEDAWAR⁴⁸ showed that dying and dead homografts were heavily infiltrated by lymphocytes. TOOLAN⁴⁹ later demonstrated that the destruction of transplanted tumors was always accompanied by a lymphocytic infiltration around the transplant and that the draining lymph node became hyperplastic. Reactions within the lymphoid system following the skin homotransplantation were studied by many investigators⁵⁰⁻⁵². They found hypertrophic draining lymph nodes containing large pyroninophilic lymphoid cells but no changes in distant lymph nodes.

On the other hand, the studies of DEMPSTER⁵³ and SIMONSEN⁵⁴ on the kidney, of DAMMIN⁵⁵ on the spleen, and of DARCY⁵⁶ on the submandibular gland are noteworthy in that plasmocytic reactions are prominent within these grafts, whereas in most other types of homografts, lymphocytic reactions predominate. The anomalous behaviors of those homografts in eliciting predominantly plasmocytic reactions have not been understood.

Although the draining lymph node consistently undergoes the most profound morphologic changes, doubtlessly, the contralateral and other lymphoid masses

are soon involved. These findings are supported by MEDAWAR³⁷, and a possibility is suggested that some immunologically competent cells may circulate in blood, be deposited in other lymphoid areas, and proliferate there. Further, it is demonstrated by the studies that the immunologically competent cells appear in the peripheral blood during immunization⁴⁸. More recently, ANDRÉ and co-workers⁵⁰ reported a systematic, long term study of the morphologic responses of the lymphoid system to the first and the second set skin homografts of rabbits. Their study demonstrated that the hemocytoblast, which arose following a variety of antigenic stimuli, also appeared after the application of homografts, and that transplantation immunity was accompanied by a proliferative response which, although it began locally, gradually spread involving distant lymph nodes and spleen. However, outbred rabbits were used in their study, seemingly in disregard of the histocompatibility system and the humoral aspects. Consequently, the significance of the plasmocytic reactions are interpreted merely on the basis that the plasmocytic proliferations of the lymphoid system are more predominant in the second set skin homografts than in the first set skin homografts.

The present study demonstrates that the plasma cell, which arises following various heterologous antigenic stimuli, also appears after the injection of homologous cells into the mouse differing in H-2 loci, and that the plasmocytic responses are obviously more predominant in the strain combination with the major histocompatibility differences than in the strain combination with the minor histocompatibility differences. In other words, the predominant plasmocytic responses with formation of humoral antibodies depend presumably upon the extent which the lymphoid system of the recipient recognizes the homologous donor tissues as not-self. It is also suggested by our study that the indispensable condition of producing humoral antibodies underlies in the relative differences of the homologous antigenicity between the donor and the recipient and not in the degree of sensitization. So far as our experiment is concerned, the D^k in H-2 loci is not so strong in antigenicity as it does not elicit plasmocytic responses sufficient for the formation of humoral antibodies in C3H strain mouse.

It seems that the cells of lymphocyte series and the cells of plasma cell series may possess some different thresholds recognizing homologous antigens as not-self, and that the threshold of the cells of plasma cell series may be higher than that of the cells of lymphocyte series. Consequently, the lymphocytes having cell-bound antibodies may be the basic barrier to homotransplantation. Further, the plasma cells may play an additional role in some homograft rejections through the humoral antibodies which they elaborate, although the direct participation of the plasma cells to the actual mechanism of homograft rejection is still obscure.

The present study again makes it clear that the morphologic indications of

transplantation immunity, which begin as an almost exclusively regional effect, eventually develop into a generalized response.

SUMMARY

For the purpose to clarify the relationship between production of humoral antibodies and cellular reactions of the lymphoid system to allogeneic-transplanted cells in mice, a study on cross sensitization was carried out between inbred A (H-2^a) and C3H (H-2^k) strain mice. The median survival time of skin of C3H transplanted to A (C₃H-to-A) was 14.1 ± 1.4 days, and of A transplanted to C₃H (A-to-C₃H) was 11.8 ± 1.6 days.

Repeated A cell injections to C3H induced the formation of humoral antibodies, whereas the C3H cell injections into A did not. In A-to-C3H and C3H-to-A combinations, the immunization induced an increase in white blood cell number in circulating blood successively with the repetition of the antigen injection, and organ weights increased in thymus, spleen, and liver but not in kidney. Weight increases in the organs of A treated with C3H cell injection were less in extent, comparing to those of C3H treated with A cells. Histologic observations revealed that the cellular proliferation in the lymphoid system including plasmocytic responses were obviously predominant in the C3H treated with A cells comparing to those in the A treated with C3H cells. Hemocytoblasts also increased during the immunization in both cases showing no significant differences between the two series of experiments. These cellular reactions were observed not only in the draining lymph nodes but also in the generalized lymphoid tissues.

The results of the present study suggest that the definitive factor for producing humoral antibodies is in the differences of the homologous antigenicity between the donor and the recipient but not in the degree of sensitization, and the D^k in H-2 loci is not so strong in antigenicity as to elicit sufficient plasmocytic responses for the formation of humoral antibodies in C3H strain mouse.

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