

Histologic Studies of Lymphoid Tissues in Mice Treated with Immunosuppressive Agents to Prolong Skin Allografts

by

MASAO ARAKAWA

From the Second Surgical Division, Kyoto University Medical School

(Director : Prof. Dr. CHUJI KIMURA)

Received for Publication July 10, 1968

INTRODUCTION

To prolong the survival of allografts in animals, many immunosuppressive procedures are employed. Immunosuppressive drugs, X-irradiation, thoracic duct drainage, injection of heterologous immune serum, administration of excessive antigen, thymectomy, and inoculation of lymphoid cells from the donor strain are used separately or together with one another. Some of these methods produce a complete tolerance to allograft survival, while others give rise only to a short term prolongation of its survival.

In this study two immunosuppressive drugs, Methotrexate (Amethopterin; 4-amino-N¹⁰-methyl pteroylglutamic acid; MTX), and Endoxan (Cyclophosphamide; N, N-bis (β -Chlorethyl) -N', O-propylene phosphoric acid ester diamide; EX), and immune plasma (goat antimouse lymphocyte plasma; GAMLP) are used. The relationship between the prolongation of allograft survival and the degree of histological change in the lymphoid tissues is examined. The purpose of the present experiments are to investigate the following problems; (1) What is the appearance of the lymphoid systems of animals retaining skin allografts in mice treated with MTX, EX, and GAMLP respectively? (2) What histologic changes occur in the lymph nodes and spleen of mice rejecting skin allografts although treated by continuous administration of MTX, EX, and GAMLP? (3) What is the difference in morphologic appearance of lymphoid systems in mice bearing the skin allografts when treated with the above mentioned immunosuppressive agents?

MATERIALS AND METHODS

Animals Mice, from a colony maintained by the Animal Center Laboratory, Faculty of Medicine, Kyoto University, were used in this study. All recipients were 10-20 weeks old A/Jax (H-2^a) males and all donors were adult C57BL/6J (H-2^b) females within the weight range of 20-35 grams. For antiplasma preparation, A/Jax, C57BL/6J, C3H/He, and ICR-JCL/T mice were used. All mice received a diet of Oriental Chow and water *ad libitum*. Two randomly bred white female goats weighing approximately 50 kg were used for preparation of GAMLP. Goats were kept in separate pens and fed a mixture of bran and bean cake and, hay with water and mineral lick *ad libitum*.

Skin grafting procedure Full-thickness suprapannicular skin allografts were performed from a donor (C57BL/6J) to 3 or 4 recipients (A/Jax)⁴¹ Three to four full-thickness discs of skin (15 mm in diameter) were routinely taken from the abdominal wall of the donor. A single full-thickness disc of skin was transplanted to the bed prepared superficial to the panniculus carnosus muscle, on the right side of the thorax. The grafts were covered with dressings of Torex* and gauze swabs, and then kept in position with plaster bandages for 7 days. Dressings were removed on the 7th postoperative day, and grafts were followed daily by visual and tactile inspection until the rejection was complete. Skin grafts were performed under aseptic conditions and with general anesthesia. Anesthesia was induced by intraperitoneal injection of Nembutal**. Any graft considered to have less than a 10% survival of epithelium was scored as completely destroyed.

Preparation of antiplasma GAMLP was prepared as follows: Repeated intravenous injection of lymph node cell suspensions in physiologic saline were used for the first inoculation of antigen and the later booster injections. Cervical and axillary lymph nodes of A/Jax, C3H/He, C57BL/6J, and ICR-JCL/T mice were excised with sterile techniques and crushed between two ordinary glass slides in a EDTA phosphate buffer saline (ph 7.2). Large particles of tissues were removed by passing the material through a double thickness of gauze. The cell suspensions were washed three times in saline. Each goat received these cell suspensions intravenously on 3 successive days to give a total dose of approximately 5×10^8 lymph node cells per goat. In addition, after 4 days interval, these procedures were repeated twice. The booster injections were given intravenously on 3 successive days for a total dose of 4×10^8 per goat per monthly interval. After such booster injections, 100 ml of blood were obtained from each goat by jugular vein puncture on the 6th, 7th, and 8th respectively. Sodium citrate was used for anticoagulant. The plasma was separated by centrifugation, pooled and stored at -20°C without the addition of antiseptic agents. The plasma was not de complemented for *in vivo* use. It was found unnecessary to absorb the plasma with mouse erythrocytes prior to use and this step was omitted in the study¹²⁾.

Lymphoagglutination Lymph node cell suspensions were prepared as described above. The cells were washed and diluted to final concentration of 6×10^8 cells per ml in saline. The plasma was heated at 56°C for 30 minutes in order to inactivate the plasma complement. One ml of the cell suspension and one ml of the serial twofold dilutions of de complemented GAMLP in saline were mixed in the test tubes (13×150 mm) and then incubated at 37°C for 2 hours. After mixing these suspensions were dropped onto the glass slides to examine microscopically for agglutination.

Immunosuppressive agents The immunosuppressive agents used were MTX, EX, and GAMLP. All of the immunosuppressive treatments were initiated at the time of grafting and continued until sloughing of the grafts occurred or animals died. These agents were administered by intraperitoneal injections at 24 and 48 hours. MTX was prepared weekly in saline containing 5 mg per ml and preserved in refrigerator at 4°C EX was prepared immediately before use in saline containing 20, 10, 5, 2.5, 0.6, and

* Non-adhesive silicone gauze, Sankyo Co., Ltd., Tokyo, Japan.

** Abbott Laboratories, North Chicago, Ill., U. S. A.

0.3 mg per ml respectively. Each batch of prepared and pooled GAMLP was checked for its lymphoagglutinin titer, and stored at -20°C separately. By melting and passing through a double thickness of gauze, the flocculation of fibrin and other denatured proteins were removed and all batches of GAMLP were mixed and then used in this study. The controls employed in this study were administered saline (10 ml/kg/day) or normal goat plasma (GNP; 0.25 ml/mouse/day).

Histology A/Jax mice bearing transplants of C57BL/6J skin were killed at 7 days interval after grafting in each schedule. Lymph nodes and spleen were excised and fixed in Carnoy's fluid or 10 % Formalin solution and embedded in paraffin wax. Sections were cut at $4\text{-}\mu$ and stained with hematoxylin and eosin and/or methyl green-pyronin if necessary.

RESULTS

Skin allografts

1. MTX In this series, 8 dose schedules were utilized. The results are shown in Table 1. (a) The maximal prolongation of allograft survival was obtained when MTX was used in Group VII (25 mg/kg/48 hrs), however, this dosage showed a high mortality. (b) In Group II (2.5 mg/kg/24 hrs) and Group IV (5 mg/kg/24 hrs), there was remarkable prolongation of allograft survival with low mortality. This prolongation of allograft survival was nearly comparable to Group VII. (c) In Group III (5 mg/kg/48 hrs) and Group V (10 mg/kg/24 hrs), all mice were alive, however, prolongation of allograft survival was worse than in Group II, IV and VII. (d) In Group VI (10 mg/kg/24 hrs) and Group VIII (25 mg/kg/24 hrs), all mice died with intact grafts and mean survival of mice was shorter than that of the allografts in the control (Group I).

Table 1 Mean Survival Time of Skin Allografts in Mice Treated with Methotrexate

Group	Dose mg/kg	No. of Total Mice	Mortality %	Successful Grafts			Death of Mice		
				No. of Mice	Mean Survival Days	Range Days	No. of Mice	Mean Survival Days	Range Days
I	*	13	0	13	11.5	8-14			
II	2.5/24 hrs	10	0	10	22.4	17-26			
III	5/48	12	0	12	17.5	16-21			
IV	5/24	11	0	11	24.3	21-30			
V	10/48	12	0	12	19.5	16-23			
VI	10/24	12	100				12	8.4	7-10
VII	25/48	12	75	3	24.6	24-26	9	17.2	13-21
VIII	25/24	12	100				12	5.8	5-7

* saline

2. EX In this series, 9 dose schedules were used. The results are shown in Table 2. (a) In the dosage ranging from 25 to 50 mg/kg/24 hrs (Group VI and VII), all mice died from toxic effects of the drug with intact grafts, however, there was marked prolongation of allograft survival. (b) In the dosage ranging from 100 to 200 mg/kg/24 hrs (Group VIII and IX), the mean survival of mice was shorter than that of the

allografts in the control (Group I). (c) In the dosage ranging from 3 to 12.5 mg/kg 24 hrs (Group II, III, and V) and 10 mg/kg 48 hrs (Group IV), the mortality of mice and the mean survival of allografts decreased. (d) When the dosage decreased from 12.5 mg/kg 24 hrs to 3 mg/kg 24 hrs, the mean survival of allografts remained almost at the same level.

Table 2 Mean Survival Time of Skin Allografts in Mice Treated with Endoxan

Group	Dose mg/kg per 24 hrs	No. of Total Mice	Mortality %	Successful Grafts			Death of Mice		
				No. of Mice	Mean Survival Days	Range Days	No. of Mice	Mean Survival Days	Range Days
I	*	10	0	10	10.3	8-12			
II	3	12	0	12	14.8	11-18			
III	6	12	0	12	14.3	11-18			
IV	10**	8	0	8	11.9	11-14			
V	12.5	12	8.3	10	15.9	11-19			
VI	25	12	100				12	34.2	26-46
VII	50	12	100				12	31.3	22-42
VIII	100	10	100				10	12.2	8-18
IX	200	12	100				12	8.2	5-10

* saline ** mg/kg/48 hrs

3. GAMPLP The first set grafting of 5 dose schedules and the second set grafting of 2 dose schedules were examined. The results are shown in Table 3. (a) The longest mean survival of allografts was observed in Group IV (0.5 ml/24 hrs). (b) In terms of mean survival of mice, Group V (1.0 ml/24 hrs) survived with successful grafts longer than Group IV. (c) When the dosage of GAMPLP was decreased from 0.5 to 0.25 ml/24 hrs (Group IV and III), the mortality was also decreased to a level which was almost one third that of the former. On the other hand, the mean survival of allografts was decreased slightly to a level which was about two third of the former. (d) In the second set grafting, the survival of allografts was prolonged. This prolongation was shorter than

Table 3 Mean Survival Time of Skin Allografts in Mice Treated with GAMPLP

Group	Dose ml/hrs	No. of Total Mice	Mortality %	Successful Grafts			Death of Mice		
				No. of Mice	Mean Survival Days	Range Days	No. of Mice	Mean Survival Days	Range Days
1st set	I	0.25/24*	9	22	7	12.4	10-17		
	II	0.25/48	10	0	10	14.3	12-15		
	III	0.25/24	15	20	12	23.1	15-41		
	IV	0.5/24	9	67	3	28.0	21-39	6	18.8
	V	1.0/48	9	78	2	23.5	21-26	7	28.1
2nd set	VI	None	10	0	10	7.0	7		
	VII	0.25/24	6	0	6	9.3	8-10		

* normal goat plasma

that in the first set grafting.

Gross findings

In morbid anatomy, the spleen was highly atrophic in the early stage of treatment with MTX and EX, but enlarged considerably in the later stages. On the contrary, in the early stage of the treatment with GAMLP, the spleen was almost constant in size, however, the late survivors showed enlarged one. The lymph nodes were atrophic during treatment with MTX and EX. In the treatment with GAMLP, enlargement of the lymph nodes was seen in the early stage, and this enlargement was observed thereafter. Intraperitoneal injections of GAMLP caused aseptic inflammation of peritoneal cavity, with the intraperitoneal viscera forming a conglomeration. This inflammation was considered to be the result of GAMLP to antiGAMLP reaction in the peritoneal cavity. In contrast, such a condition was not seen in the controls which were treated with normal goat plasma. In this group, the most conspicuous finding was noticeable enlargement of all lymph nodes. Also, an enlargement of the spleen was observed in most of the cases.

Microscopic observation

Host mice were killed at 7 days interval after grafting in each schedule. They showed the following histologic changes

1. MTX

Spleen: In all cases, the spleen showed a significant atrophy with many preserved lymphocytes in the follicles. In mice which survived over 4 weeks with successful grafts, the lymphocytes remained in the white pulp, however, there was a decrease in number. The plasmacytes disappeared in all cases (Fig. 1).

Lymph nodes: Lymphoid follicles were seen in the cortical areas of the nodes. No

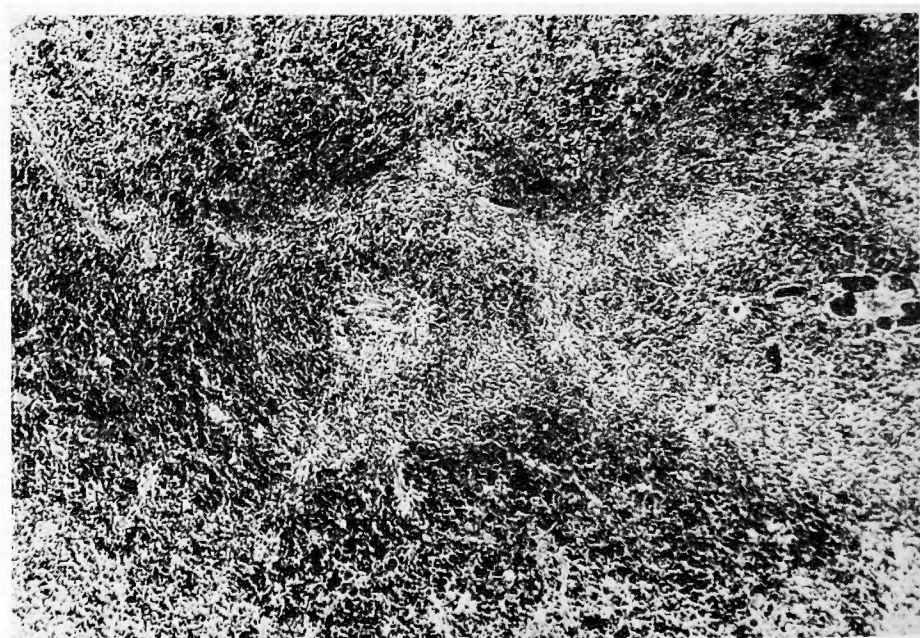


Figure 1 Spleen from the mouse treated with MTX (15mg/24hrs) for 4 weeks. Showing a follicular atrophy, lymphocytes remain and decrease in number. $\times 40$.

plasmacytes were observed (Fig. 2).

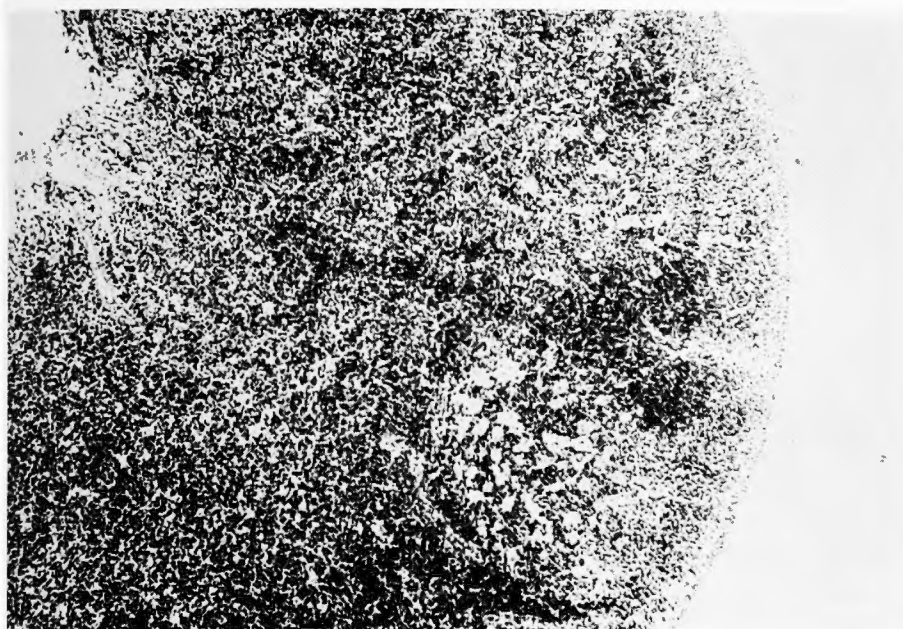


Figure 2 Lymph node from the mouse treated with MTX (25mg/24hrs) for 1 week. Decreasing in number, lymphocytes remain. $\times 40$.

2. EX

Spleen: In Group VI, VII, VIII, and IX (25, 50, 100, and 200 mg/kg 24 hrs), white pulps were atrophic with the lymphocytes diminished in number and then replaced by masses of reticulum cells. The proliferation of reticulum cells seen in the peripheral areas of the white pulps and red pulps (Fig 3, 4). The obliteration of follicles and the proliferation of red pulps were observed sequentially. These phenomena resulted in an increase in the size of the spleen. The enlargement of the spleen was seen at a late stage in these groups. In Group II, III, IV, and V (3, 6, 12.5 mg/kg/24 hrs and 10 mg/kg/18 hrs respectively), the follicular structure with germinal centers remained. A congestion and reticular proliferation in the red pulps were observed. (Fig. 5, 6).

Lymph nodes: In the cortical areas, the lymphoid population was observed. However, the width of these areas was decreased. In the medullary areas, scatterings of lymphocytes and proliferation of reticulum cells was observed.

3. GAMLP

Spleen: In the control (Group I), the size of follicular structure with germinal centers was enlarged and it was replaced by masses of proliferating lymphocytes. The areas of white pulps around the central arterioles were occupied by masses of tightly packed mature and immature plasmacytes (Fig. 7). Zones of lymphocytes enclosed masses of plasmacytes. Also, the proliferation of such plasmacytes was seen in the red pulps. Congestion was variable in a degree, and was sometimes quite significant. The histologic findings of Group III, IV, and V (0.25, 0.5 ml 24 hrs and 1.0 ml 18 hrs) were summarized as follows: In the early stages of treatment, the obliteration of lymphoid follicles and the replacement

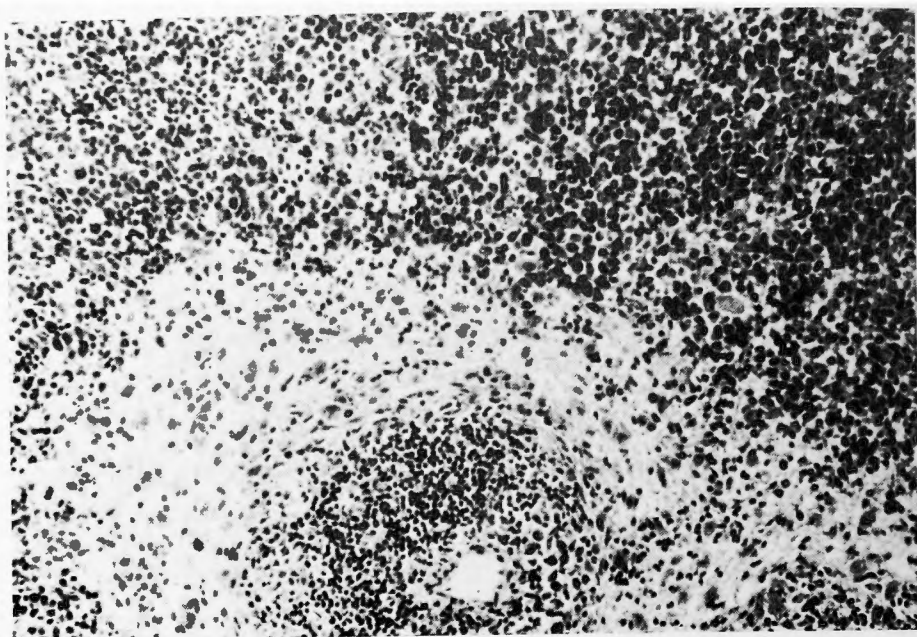


Figure 3 Spleen, treated with EX (25mg/24hrs) for 3 weeks. Marked depletion of lymphocytes and massive proliferation of reticulum cells. $\times 100$.

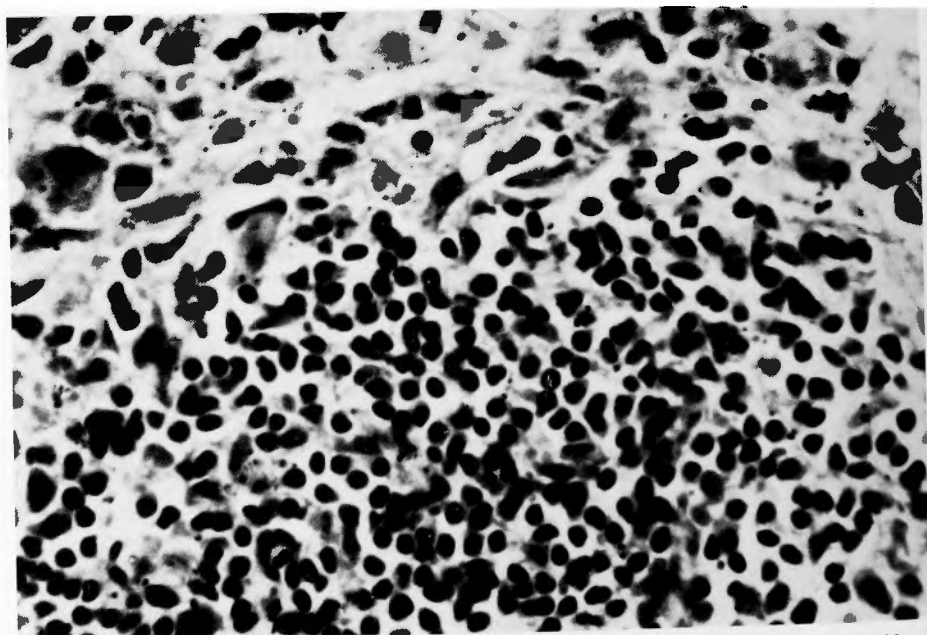


Figure 4 High power view of a portion shown in Fig. 3. Lymphocytes are pyknotic. $\times 400$.

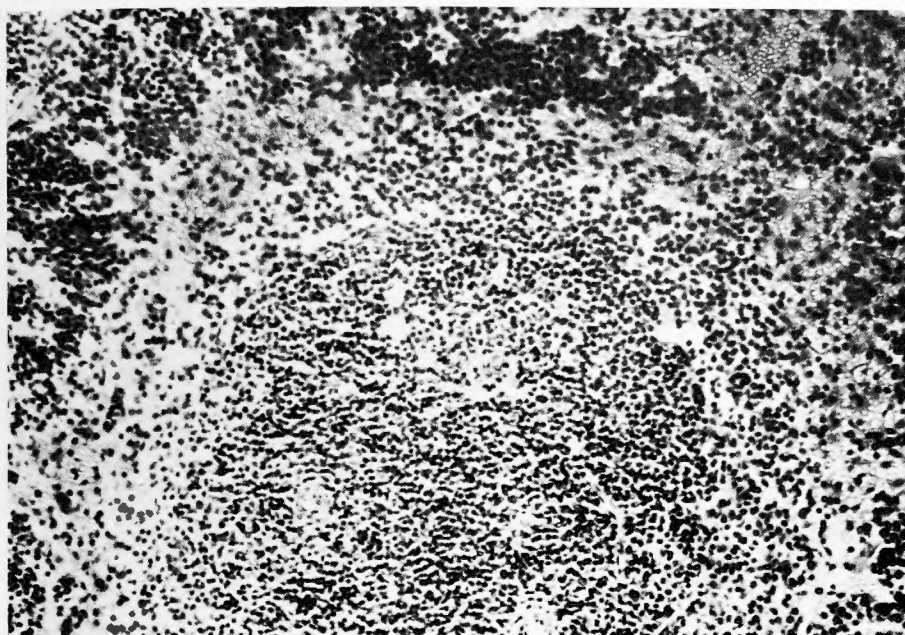


Figure 5 Spleen, treated with EX (10mg/48hrs) for 2 weeks lymphocytic depletion is scarcely seen. $\times 100$.

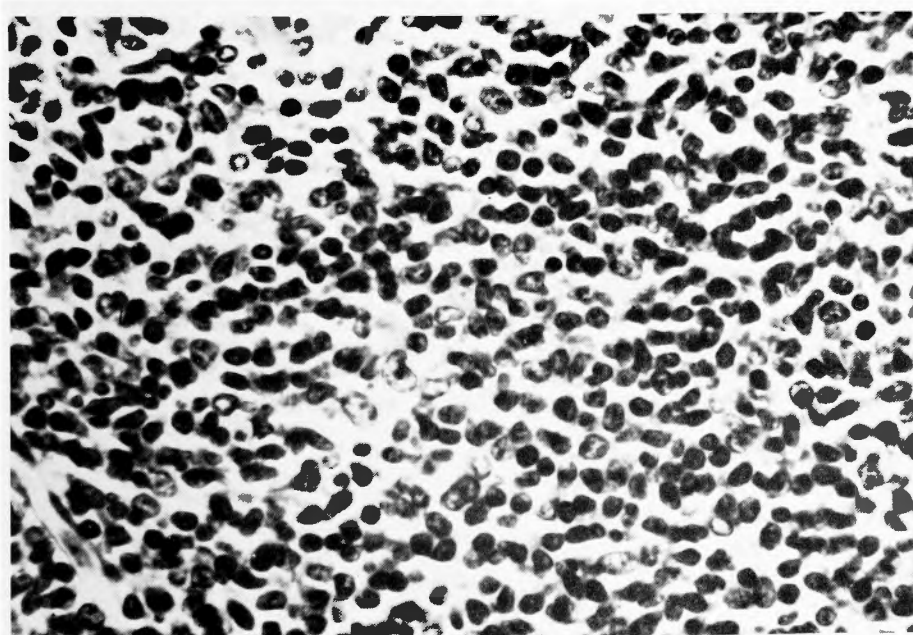


Figure 6 High power view of a portion shown in Fig.5. $\times 400$.

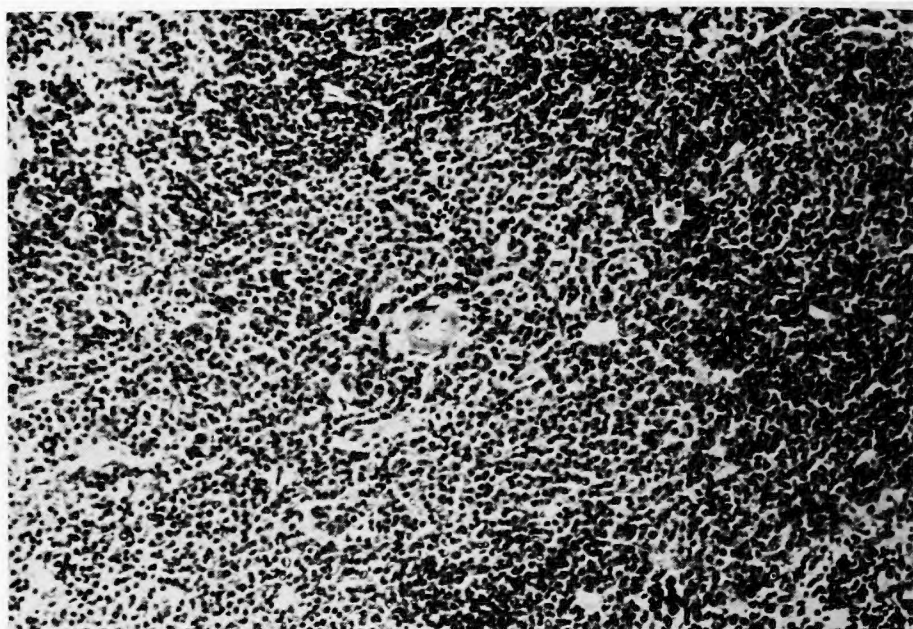


Figure 7 Spleen, treated with normal goat plasma (0.25ml/24 hrs.). Enlargement of the lymphoid follicle and proliferation of immature plasmacytes around the central arterioles. $\times 100$.

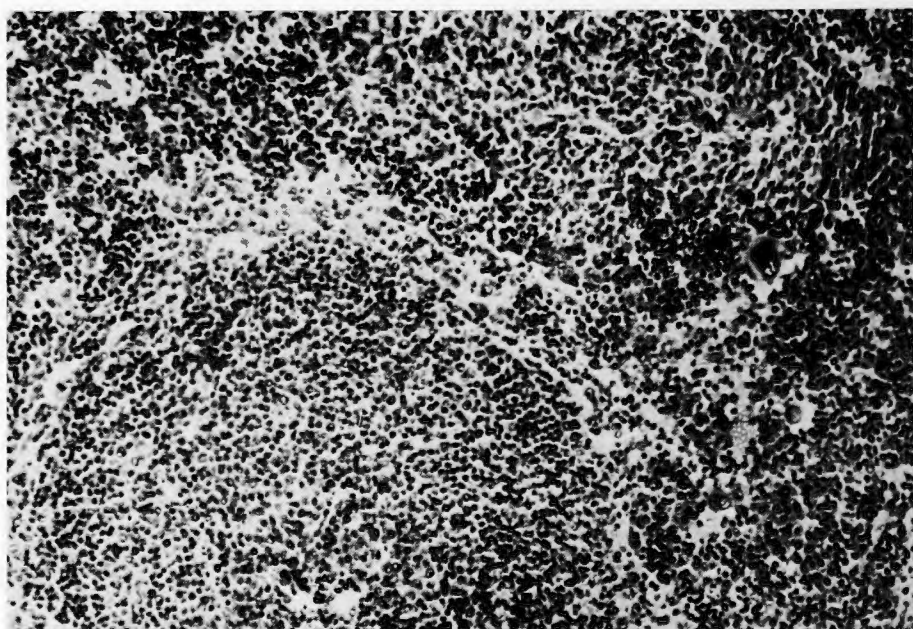


Figure 8 Spleen, treated with GAMLIP (1.0ml/18hrs) for 3 weeks. Marked depletion of lymphocytes and masses of immature plasmacytes in the white pulp. $\times 100$.

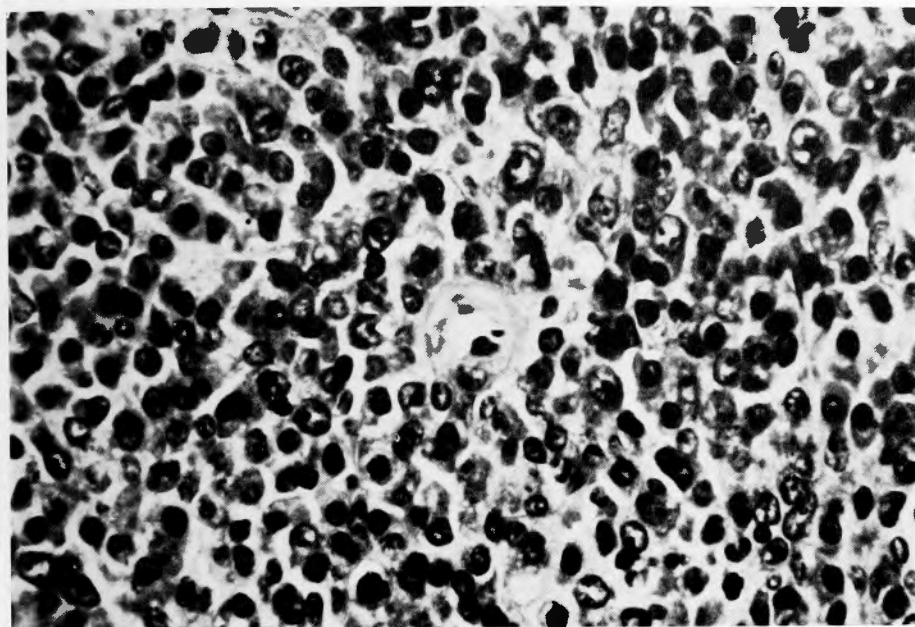


Figure 9 High power view of a portion shown in Fig. 8. Showing the immature plasmacytes around the central arteriole. $\times 400$.

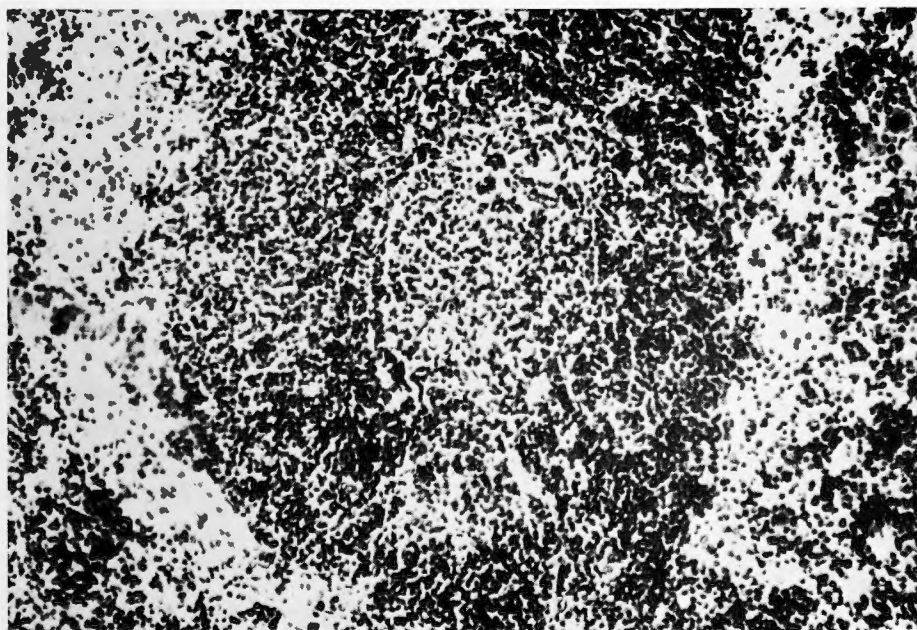


Figure 10 Spleen, treated with GAMLIP (0.25ml/24hrs) for 3 weeks, showing repopulation of the lymphocytes in the peripheral areas of the white pulp, massive proliferation of the immature plasmacytes remains. $\times 100$.

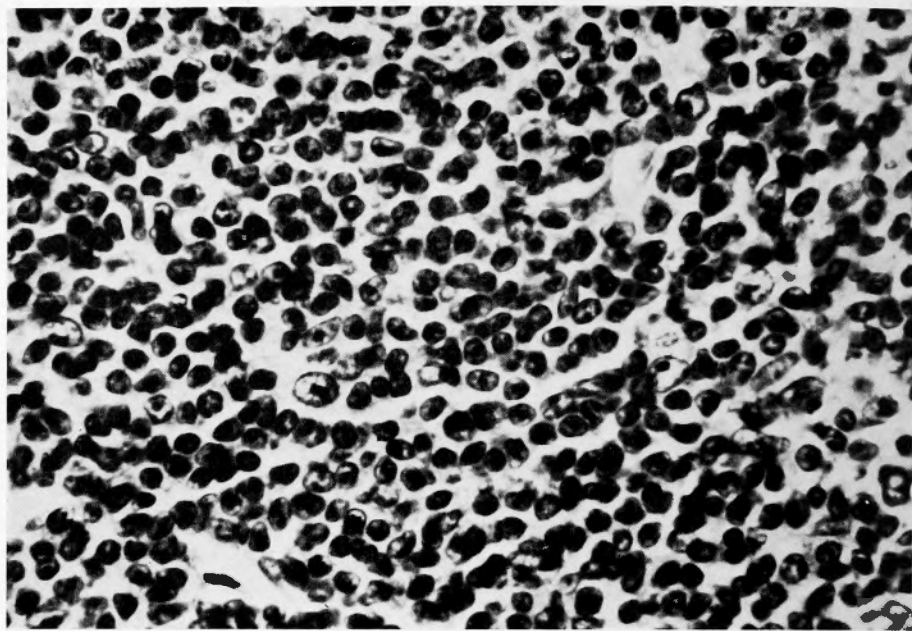


Figure 11 High power view of a portion shown in Fig. 10. Showing proliferation of the lymphocytes. $\times 400$.

with masses of proliferating immature plasmacytes was observed (Fig. 8, 9). Approximately 1 to 2 weeks later, treatment with GAMLP was continued, however, the proliferation of the lymphocytes appeared and resulted in the formation of the new lymphoid follicles. These follicles were formed outside the masses of mature and immature plasmacytes which occupied the areas around the central arterioles of the white pulps. This repopulation of lymphocytes was initiated by the beginning of the 3rd week after grafting (Fig. 10, 11).

Lymph nodes: In all cases, lymphocytes diminished in number and existed in the cortical areas. In the medullary areas, the marked proliferation of mature and immature plasmacytes was observed, with progressive expansion of the lymph nodes caused by the proliferation of the plasmacytes.

DISCUSSION

Only a few morphologic observations have been made to determine which cell types are affected by the immunosuppressive agents^{11,2)}. SCHWARTZ mentioned that transplantation immunity is disturbed by MTX when it blocks the action of dehydrofolic reductase, the enzyme required to convert folic acid to its active form of folinic acid¹³⁾. A cell whose supply of folinic acid is "choked off" by MTX will be unable to construct nucleic acid and proteins. TURK *et al* reported that MTX inhibited the development of delayed hypersensitivity in guinea pigs¹⁶⁾. Unlike 6 Mercaptopurine (6-MP), it failed to inhibit the appearance of hemocytoblasts in the local lymph nodes. On the other hand, plasmacytes were blocked. MTX appears to block the development of "immunologically committed" small lymphocytes and plasmacytes from hemocytoblasts, while EX inhibits the

growth of hemocytoblasts *per se*. MTX has been tried in various attempts to retard allograft rejection³³⁸, and utilized in attempts to prolong the survival of organ grafts⁵³¹⁴. In the present study using MTX, it has been shown that the disappearance of the small lymphocytes occurred gradually, and that the lymphoid structures were preserved for 3 to 4 weeks. The duration of MTX administration was correlated with the prolongation of allograft survival and administration of drug over a 24 hr period had better results than that administered over a 48 hr period. However, this phenomenon was not correlated with the histologic changes of lymphoid tissues. The relationship of the histologic change and MTX administration periods was obscure in the present study.

EX interacts directly with protein and DNA molecules, causing their denaturation. EX is a powerful lymphocytolytic agent and it may suppress the immune response by depleting the number of small lymphocytes. GOWANS *et al* have presented important evidence showing that the presence of these cells is necessary for the inhibition of the immune response⁹. Their destruction by EX would thus lead to immunologic unresponsiveness. EX prevented the appearance of hemocytoblasts¹⁶, and appeared to act by depletion of the precursor cells (small lymphocytes), and hence was effective in suppressing antibody synthesis when given before contact with the antigen. The precursor cells, most likely small lymphocytes, signal the onset of immunological commitment by transforming into hemocytoblasts which ultimately develop into plasmacytes or lymphocytes. In the present study with EX, we have obtained a marked obliteration of the lymphoid follicles and, invisibility of hemocytoblasts and plasmacytes. On the other hand, proliferation of the reticulum cells occurred with masses of reticulum cells occupying the area without lymphocytes. Disappearance of the lymphocytes was an important characteristic of EX induced tolerance in allografts. When EX was given by continuous administration in 25 mg/kg/24 hrs or more, complete drug induced tolerance was obtained, but the animals died from the side effects of EX. In these doses, lymphoid tissues showed marked depletion, and recovery was not observed. The dosage of the drug administered is critical.

Antilymphocytic serum or plasma (ALS or ALP) is the name given to an antiserum or antiplasma produced in members of one species by the injection of lymphocytes or lymphoid cells taken from members of another species. ALS or ALP is a heterogeneous mixture of antibodies directed against several antigenic species. According to the description of LEVEY and MEDAWAR¹⁰¹¹¹, ALS has the power not only to prevent or delay the onset of immunologic reactions but also to arrest reactions already in progress. This combination of characters is unique, especially since ALS is devoid of acute toxicity, and is one of the most powerful immunosuppressive agents in transplantation. LEVEY and MEDAWAR estimated hypothetically the mode of action of ALS as follows: (a) ALS acts essentially as a lymphocytolytic agent. (b) Heterologous antisera act as a competitive antigen. (c) Action through the thymus: they neutralize a humoral factor manufactured in the thymus. (d) ALS acts by preventing the recognition of antigen: ALS coats lymphocytes in such a way as to occlude their combining sites or recognition units (blindfolding theory). (e) ALS acts as a blast cell transformation. The blast cell transformation is connected with the immunological inactivation of lymphocytes (sterile activation)⁹¹¹⁰. MONACO *et al* have an opinion contrary to LEVEY *et al* in some aspects of the mode of action of ALS⁷¹¹²¹¹³. MONACO *et al* emphasize that the effect of ALS is explained on

the basis of lymphoid cell destruction.

In the present study using GAMLP, three phases of microscopic changes are observed sequentially. In the first phase, the disappearance of the lymphocytes and the appearance of the blast cells which are called hemocytoblasts or immature plasmacytes occur in both of the white splenic pulps and medullary areas of lymph nodes. In the second phase, the massive proliferation of the blast cells is characteristic in both of the white splenic pulps and the medullary areas of the lymph nodes. The lymphocytes remained unchanged in number. In the third phase, the marked repopulation of lymphocytes in the peripheral areas of the white splenic pulps is characteristic. Masses of blast cells remained unchanged in number. These histologic changes in the lymphoid tissues during the GAMLP treatment corresponded to the destruction of the skin allografts which survived against the strong difference of the H-2 locus. This phenomenon suggested that there was the development of the antiGAMLP action. ANDRE *et al* noted that the resistance against immunosuppressive drug occurred during prolonged treatment with 6-MP in rabbits²⁾. AntiGAMLP resistance may occur both in the lymphocytes and the plasmacytes of the mice which are chronically treated with GAMLP. When changes do occur in the lymphocytes, they will be of an antilymphocytolytic or antilindfolding action. In contrast, when the changes occur in the plasmacytes, they will have an antibody producing action against GAMLP. We have observed the rise of the precipitin titer to GAMLP in the mice treated with GAMLP (0.25 ml/24 hrs) for 2 weeks. We assume that the resistance against GAMLP occurs in the plasmacytes. These plasmacytes produce the antibody which has an action against GAMLP.

SUMMARY

The effects of MTX, EX, and GAMLP on the histologic responses of lymph nodes and spleen were studied in 270 mice (A/Jax). The administration of MTX, EX, and GAMLP were initiated on the day of grafting and continued until the rejection of the tissues was complete. Skin allografts were performed. When MTX was given at a dose of 5 mg/kg/24 hrs, the drug was most effective in the prolongation of skin allograft survival. The histologic changes of lymphoid tissues in this group showed moderate and progressive depletion. When EX was given at a dose of 25 mg/kg/24 hrs, the drug likewise very effective in the prolongation of skin allograft survival. However, all mice treated at this dosage of EX died from side effects of the drug. The histologic changes observed in this group showed marked and progressive lymphoid depletion. GAMLP was given at doses ranging from 0.5 to 0.25 ml/mouse/24 hrs, and was found to be effective also in prolongation of skin allograft survival. Histologic changes in this group showed lymphocyte effacement and hemocytoblast formation. Also, the proliferation of hemocytoblasts and the repopulation of lymphocytes occurred. These cells were considered to be the GAMLP-resistant cells.

ACKNOWLEDGMENTS

The author wishes to take this opportunity to express his deep appreciation to Professor Dr. CHUJI KIMURA, and Dr. RYO INOUE, who helped and encouraged him during this entire period. The author is also indebted to Drs. MAMORU YAMANE, and YOICHI OJIMA for their discussion and advice, and to Dr. MASAHISA KYOGOKU for

his counsel in regard to the pathological changes. The proficient technical assistance of Miss KIMIKO TABUCHI is gratefully acknowledged.

REFERENCES

- 1) Andre, J. A., Schwartz, R. S., Mitus, W. J. and Dameshek, W. : The morphologic responses of the lymphoid system to homografts. I. First and second set responses in normal rabbits. *Blood*, **19** : 313, 1962.
- 2) Andre, J. A., Schwartz, R. S., Mitus, W. J. and Dameshek, W. : The morphologic responses of the lymphoid system to homografts. II. The effects of antimetabolites. *Blood*, **19** : 334, 1962.
- 3) Berenbaum, M. C. : Prolongation of homograft survival in guinea pigs treated with amethopterin. *Nature*, **198** : 606, 1963.
- 4) Billingham, R. E. and Medawar, P. B. : The technique of free skin grafting in mammals. *J. Exp. Biol.*, **28** : 385, 1951.
- 5) Blumenstock, D. A., Collins, J. A., Hechtman, H. B., Thomas, E. D. and Ferrebee, J. W. : Functioning of the lung in dogs. *Ann. N. Y. Acad. Sci.*, **99** : 882, 1962.
- 6) Gowans, J. L., McGregor, D. D. and Cowen, D. M. : Initiation of immune responses by small lymphocytes. *Nature*, **196** : 651, 1962.
- 7) Gray, J. G., Monaco, A. P., Wood, M. L. and Russell, P. S. : Studies on heterologous anti-lymphocyte serum in mice. I. In vitro and in vivo properties. *J. Immun.*, **96** : 217, 1966.
- 8) Hechtman, H. B., Blumenstock, A. D., Thomas, E. D. and Ferrebee, J. W. : Prolongation of canine skin homografts by antimetabolites. *Surg. Forum*, **13** : 55, 1962.
- 9) Levey, R. H. and Medawar, P. B. : Some experiments on the action of antilymphoid antisera. *Ann. N. Y. Acad. Sci.*, **129** : 164, 1966.
- 10) Levey, R. H. and Medawar, P. B. : Nature and mode of action of antilymphocytic antiserum. *Proc. Natn. Acad. Sci.*, **56** : 1130, 1966.
- 11) Levey, R. H. and Medawar, P. B. : Further experiments on the action of antilymphocytic antiserum. *Proc. Natn. Acad. Sci.*, **58** : 470, 1967.
- 12) Monaco, A. P., Wood, M. L., Gray, J. G. and Russell, P. S. : Studies on heterologous anti-lymphocyte serum in mice. II. Effect on the immune response. *J. Immun.*, **96** : 229, 1966.
- 13) Monaco, A. P., Wood, M. L. and Russell, P. S. : Studies on heterologous anti-lymphocyte serum in mice. III. Immunologic tolerance and chimerism produced across the H-2 locus with adult thymectomy and anti-lymphocyte serum. *Ann. N. Y. Acad. Sci.*, **129** : 190, 1966.
- 14) Reemtsma, K., Williamson, W. E., Iglesias, F., Pena, E., Sayegh, S. F. and Creech, O. : Studies in homologous canine heart transplantation : Prolongation of survival with a folic acid antagonist. *Surg.*, **52** : 127, 1962.
- 15) Schwartz, R. S. : Immunosuppressive Drugs. *Progr. Allergy*, **9** : 245, 1965.
- 16) Turk, J. L. and Stone, S. H. : Implication of the cellular changes in lymph nodes during the development and inhibition of delayed type hypersensitivity. In *Cell Bound Antibody*, 51, 1963.

和文抄録

移植免疫反応抑制剤投与時の同種皮膚移植マウスにおける
リンパ組織の組織学的研究

京都大学医学部外科学教室第2講座（指導：木村忠司教授）

荒 川 正 夫

この研究の目的は3種類の異つた移植免疫反応抑制処置を行なつた際の被移植動物における 1) 移植片生着時のリンパ組織の所見、2) 移植免疫反応抑制を行なつたにもかかわらず移植片の拒絶現象のみられた動物におけるリンパ組織の所見、3) 抑制法が異なることによるリンパ組織の変化の差の3点を観察し考察を加えることにある。移植免疫反応抑制法には ① Methotrexate (MTX)、② Endoxan (EX)、③ 山羊抗マウスリンパ球血漿 (GAMLP) を用いた。A/Jax マウス270匹を Recipient とし、これの背部皮膚を一部除去してここに C57BL マウスの腹部皮膚を全層移植した。MTX、EX、GAMLP は共に移植と同時に腹腔内注射により投与を開始し移植片脱落までつづけた。移植片の生着延長を観察した。組織学的には経時的に移植片生着マウスを殺し、標本とした。移植片脱落マウスは直ちに殺して標本とした。対照には MTX 及び EX 投与群では生理的食塩水を用い、GAMLP 投与群には正常山羊血漿を用いた。次の結果を得た。

1) MTX 群

他群に比べ最もリンパ組織の変化が少く、又生着群と拒絶群の間に明かな組織学的な差をみなかつた。

2) EX 群

1回の投与量で 12.5 mg/kg/24 hrs と 25 mg/kg/24 hrs の間に限界点があり、投与量が限界点を越えると

リンパ組織においていちぢるしいリンパ球の減少がおこり、限界点以下ではリンパ球の減少は軽度である。移植片の生着もそれに応じて、限界点以上の投与量では動物は生着した移植片を持ちながら死亡する。この時の移植片の生存期間は対照に比べいちぢるしく長い。これに反し、限界点以下の投与量では拒絶現象がおこり、移植片生着期間は対照よりもやや長いと言うにすぎない。

3) GAMLP 群

GAMLPにおいては投与量の差による生着延長の差はEX群のようにいちぢるしくない。全例において拒絶現象がみられている。この群におけるリンパ組織の変化の特長は2つあり、その1つは若弱型の形質細胞のいちぢるしい増殖であり、他の1つはリンパ球の一時的な減少並びに次に起つて来る増加である。リンパ球は早期にはいちぢるしい減少をみせるが、約3週目より再び多数出現し、白色髄の辺縁部にリンパ濾胞を形成する。このリンパ濾胞の再現は移植片脱落の時期に一致している。何故にGAMLPの投与がつづくにもかかわらず、リンパ球の出現が起るかは、1つには異種血漿に対する抗体の出現のためと考える。他の1つには抗リンパ球抗体耐性のリンパ球群の出現によるものであらうと考える。