Pathogenesis of Graft-versus-Host Reaction
I. Influence of Thymectomy and Adrenalectomy on Development of of Lymphopenia[1] (36239)

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Several names, as runt disease, homologous disease and secondary disease, have been given to the syndrome produced by introduction of immunologically competent cells into a host that is unable to reject those cells. There can be no doubt that the syndrome is initiated by an immunologic graft-vs.-host reaction (GVH). Numerous reports (1–3) have described the clinical and pathological features that develop consequent to GVH reaction induced by diverse methods in several species of animals. One characteristic feature of the GVH reaction is the pathology of the host lymphoid tissue. Initially, there is marked enlargement of the spleen and lymph nodes which is followed by atrophy of the organs. By contrast, the thymus promptly atrophies without initial enlargement; this has been referred to as "immunologic thymectomy" (3).

Amelioration of GVH reaction by adrenalectomy has been reported (2). However, in our experience animals adrenalectomized prior to induction of GVH reaction die earlier than do similarly treated intact animals (4, 5). One pathologic change during GVH reaction is notably different in adrenalectomized animals as compared to intact animals. Whereas the thymus of intact animals atrophies soon after induction of the GVH reaction, the thymus of adrenalectomized animals remains large and retains its normal histologic appearance (5, 6). In addition, by removing the adrenal glands 7–14 days after induction of the GVH reaction, thymic involution already in progress is not only halted, but is reversed, so that the thymus soon regains its normal size and appearance (7). Since thymic destruction during the GVH reaction appears to be mediated by the adrenal gland secretions, the concept of "immunologic thymectomy" implying thymectomy by direct immunologic destruction seems untenable.

In consideration of the destruction of central lymphoid tissue that is attributable to adrenal gland hypersecretion, it seems likely that these secretions also contribute significantly to destruction of peripheral lymphoid tissue during GVH reaction. In the present investigation we sought to determine the relationship of the adrenal gland functions to the concomitant thymic involution and development of lymphopenia during GVH reaction.

Materials and Methods. Mice. Mice of the C3H/bi strain 6–8 months of age were used as spleen cell donors. The spleen cell recipients were (C3H × C57)F1 hybrid mice resulting from the cross between the C3H/bi and C57Bl/1 strains.

Experimental design. Thirty days before injection of cells, 25 (C3H × C57)F1 hybrid mice 1–3 months of age were thymectomized; nineteen similar animals were retained without thymectomy. Four days before induction of GVH reaction, lymphocyte counts were made on the mice of these two

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groups; these are recorded as the initial lymphocyte counts.

The following day, 3 days prior to injection of cells that induced the GVH reaction, the animals were either adrenalectomized or sham-adrenalectomized. Thus, the groups in this experiment are as follows:

Four groups of experimental (C3H × C57)F1 hybrid animals given iv 75 million spleen cells obtained from C3H strain donors

1. Eight thymectomized and adrenalectomized animals, Group TAI.
2. Five adrenalectomized animals, Group AI.
3. Eight thymectomized and sham-adrenalectomized animals, Group TSAI.
4. Five sham-adrenalectomized animals, Group SAI.

Four control groups of similar animals were given no cells:

1. Four thymectomized and adrenalectomized animals, Group TA.
2. Five adrenalectomized animals, Group A.
3. Five thymectomized and sham-adrenalectomized animals, Group TSA.
4. Four sham-adrenalectomized animals, Group SA.

Thymectomy. Thymectomies were performed on animals lightly anesthetized with ether and secured to an operating board. After cleaning the neck region with 70% alcohol a midline longitudinal incision was made in the skin and superficial fascia. This incision extended from the level of the angle of the mandible to the level of the 4th rib. The submaxillary glands were freed and retracted anteriorly, thus exposing the sternohyoid muscle attachment. The sternohyoid muscles were separated exposing the trachea and manubrium sterni. An incision was made down the center of the manubrium and sternum to the level of third rib. The bisected sternum was then retracted ventrally and laterally and the fascia overlying the mediastinum was separated longitudinally exposing the thymus. Each lobe of the thymus was aspirated, using a suction cannula to gently manipulate it free of its attachments; the site was carefully inspected for residual thymic tissue. Skin closure was accomplished by approximating the skin edges and applying 7.5 mm Michel-wound clips.

Adrenalectomy and sham-adrenalectomy. Three days before infusion of spleen cells all prospective recipient and control animals, lightly anesthetized with ether, were subjected to either adrenalectomy or sham operation via a dorsal midline incision through the skin and an incision in the muscular plane lateral to the midline over the anterior margin of each kidney. For adrenalectomy, the exposed adrenal glands were grasped with a forceps and excised; sham-adrenalectomy was performed in a similar manner, but the adrenal glands were not excised. Skin closure was accomplished using 7.5. mm Michel-clips; the muscular plane incision closed spontaneously within 7 days.

Spleen cell preparation and injection. Donor animals were sacrificed by exposure to a high concentration of carbon dioxide gas; their spleens were immediately removed and placed in cold lactate–Ringer's solution. The spleens were minced into a Potter-Elvehjem homogenizer containing cold lactate–Ringer's solution. A loose fitting pestle was used to gently express the cells from the capsule; a tighter fitting pestle was used to separate the cells from the pulp. The cell suspension was drawn into a syringe through a 27 gauge needle and expressed into a clean moistened beaker. About 10 units of heparin per donor spleen was added to the suspension and a cell count was obtained in the usual manner for counting leukocytes. Within one hour after preparation a spleen cell suspension containing 75 million cells was injected into the lateral tail vein of (C3H × C57)F1 hybrid mice lightly anesthetized with ether.

Lymphocyte counts. Blood samples were obtained from individual mice of each group 4 days before injection of cells and 6, 12, 18, and 24 days after cell injection. Leukocyte counts and differential counts were obtained by standard methods; from these values the lymphocyte counts were calculated.

Statistical evaluation. Mean lymphocyte counts were evaluated for statistically significant differences by intragroup and intergroup comparison using the Student t test.

Animal care. All animals were kept in a

TABLE I. Mean Lymphocyte Counts of Variously Manipulated (C3H × C57Bl/1)F1 Hybrid Mice During Graft-vs.-Host Reaction.

Group[a]	Days before reaction	Days after induction of reaction			
	—4	6	12	18	24
TAI	17,730	14,780	12,140	10,720	11,320
	900[b]	2000	1780	1930	1290
AI	13,290	8530	3470	3170	8710
	710	1950	1350	1310	1850
TSAI	17,730	6930	3820	2000	4200
	900	1650	1370	610	500
SAI	13,290	8170	1670	1130	4410
	710	1430	1040	370	1420
TA	17,730	14,360	15,620	12,790	13,670
	900	3060	3820	2830	2260
A	13,290	15,210	15,530	12,230	15,780
	710	1460	3320	2870	1430
TSA	17,730	11,840	12,750	10,920	12,920
	900	1660	1620	1770	1810
SA	13,290	16,080	8300	7150	12,870
	710	2670	1650	1360	2720

[a] In the group designations the following symbolism is used: T, animals were thymectomized; A, animals were adrenalectomized; SA, animals were sham-adrenalectomized; I, animals were injected intravenously with 75 million C3H spleen cells. Thus, Group TSAI was thymectomized, sham-adrenalectomized and injected with cells.

[b] Standard error of the mean.

temperature and humidity controlled room prior to and during the observation period. The rooms were illuminated by fluorescent lights turned on at 6:00 AM and off at 6:00 PM. Not more than five animals were housed in each clear plastic box with 84 square inches of floor space. All animals were fed Purina Lab Chow *ad lib.* Adrenalectomized animals were provided 0.9% sodium chloride drinking water; other animals were supplied tap water. Boxes and water bottles were changed and sterilized weekly.

Results. Lymphocyte counts are recorded in Table I and Fig. 1. The initial mean lymphocyte count obtained from the group of thymectomized animals was significantly greater than the count obtained from the group of nonthymectomized animals (Fig. 1). The mean lymphocyte count of the thymectomized group was 17,730/mm^3, compared to a lymphocyte count of 13,290/mm^3 in the nonthymectomized group (Table I).

Except for the animals subjected to both thymectomy and adrenalectomy, Group TAI, the recipients of spleen cells developed lymphopenia soon after induction of GVH reaction. Six days after injection of cells, the lymphocyte count of Group TAI did not differ significantly from the initial value or from the lymphocyte count obtained from the noninjected control animals, Group TA. By contrast, the nonthymectomized groups, Group AI and Group SAI, and the thymectomized and sham-adrenalectomized animals of group TSAI had lymphocyte counts significantly lower than the initial lymphocyte count. Further, the lymphocyte counts of these three groups, Group AI, SAI, and TSAI, were lower than the lymphocyte counts of their controls, respectively, Group A, Group SA, and Group TSA.

The degree of lymphopenia, in Groups AI,

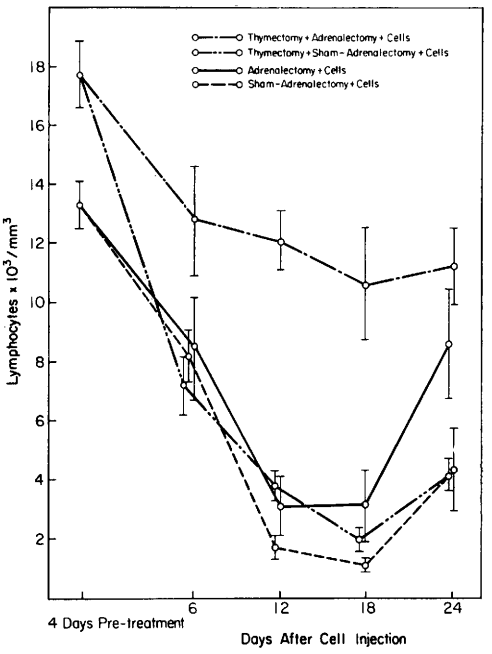

FIG. 1. Mean lymphocyte counts of variously manipulated groups of (C3H×C57Bl/1)F1 hybrid mice before and at intervals after induction of GVH reaction by injection of spleen cells from adult C3H strain donors. Range of values from noninjected control groups is 7150–15,780 (see Table I). Vertical bars represent the standard error of the mean values.

SAI, and TSAI, was greatest on the twelfth and the eighteenth post-induction days; the mean lymphocyte counts ranged between 1100/mm^3 and 3800/mm^3. These lymphocyte counts were significantly lower than the lymphocyte counts obtained from any of the control groups. In marked contrast, the lymphocyte counts obtained from Group TAI the twelfth and eighteenth post-induction days were, respectively, 12,140/mm^3 and 10,720/mm^3. These counts were not significantly different from those of any of the control groups.

The twenty-fourth post-induction day the severity of the lymphopenia observed earlier in Groups AI, SAI and TSAI had diminished; at this time the lymphocyte counts of these groups were, respectively, 8710/mm^3, 4410/mm^3 and 4200/mm^3. The lymphocyte counts of these groups were significantly lower than the lymphocyte counts of their control groups. The lymphocyte counts of Groups TSAI and SAI were significantly lower than the lymphocyte counts of all the control groups and also were lower than the lymphocyte count of Group TAI on the 24th post-induction day. By contrast, the lymphocyte count of Group TAI, on the 24th post-induction day, was not significantly lower than the lymphocyte counts of the control groups. The difference between the lymphocyte counts of Group TAI and Group AI, on the 24th post-induction day was not statistically significant.

Discussion. These data demonstrate that thymectomy and adrenalectomy performed on the recipients before induction of GVH reaction prevents development of severe lymphopenia usually associated with GVH reaction. Performance of either thymectomy or adrenalectomy before induction of the reaction did not prevent development of lymphopenia. However, there was a slight suggestion that adrenalectomy reduced the severity of lymphopenia and adrenalectomized animals seemed to recover from lymphopenia more rapidly than did the nonadrenalectomized animals.

Removal of the adrenal glands would obviate lymphocyte destruction mediated by adrenal gland secretion consequent to the GVH reaction. Adrenalectomy alone did not greatly alter the development of lymphopenia during the GVH. Nonetheless, the magnitude of lymphopenia was slightly decreased and its duration was appreciably shortened by adrenalectomy. More dramatic was the marked reduction of lymphopenia in thymectomized mice experiencing a GVH reaction. The mechanism underlying this influence of thymectomy deserves consideration. Van Putten (13) reported that thymectomy of recipients prior to induction of GVH by injection of bone marrow cells from parental donors reduced mortality from secondary disease. Logically this author attributed this effect of thymectomy to elimination of an essential differentiative influence of thymus on the injected marrow cells. In our experiments fully differentiated immunologically competent spleen cells were used to induce the GVH. Thus it seems likely that the influ-

ence of thymectomy on lymphopenia in GVH, which we report must be reflecting an influence on host cells. Thymectomy in adult mice results in a progressive decline of certain immunologic functions (8–10) and a decrease in the population of T cells subserving the thymus-dependent functions (11, 12). Perhaps the influence of thymus on this component of the lymphoid tissue accounts for the alteration of the influence of GVH on lymphocyte numbers.

The lymphocyte population is composed of a proportion of thymus-dependent lymphocytes and a proportion of thymus-independent lymphocytes. Thymectomy performed on adult animals results in diminution of the proportion of thymus-dependent lymphocytes and a compensatory increase in the proportion of thymus-independent lymphocytes. Adrenal gland hyperactivity consequent to the GVH reaction results in destruction of both thymus-dependent and thymus-independent lymphocytes. If we postulate that the immunological assault of the GVH reaction is primarily directed against thymus-dependent lymphocytes, while the thymus-independent lymphocytes are relatively insusceptible to the immunologic component of GVH the observations made in this study can be explained.

The thymectomized-sham-adrenalectomized animals became lymphopenic since the thymus-independent lymphocytes, not the primary target of the immunologic assault, were susceptible to the action of the adrenal gland secretions. Although the GVH-initiated by the experimental procedure used was sublethal for adrenalectomized animals, it was of sufficient vigor to provoke an adrenal gland hyperactivity reflected in the drop in circulating lymphocytes. This influence of the adrenal on thymus involution during GVH has been documented in our earlier papers (5, 6).

The non-thymectomized, sham-adrenalectomized group of animals become markedly lymphopenic during the GVH because they lose lymphocytes of both thymus-dependent and thymus-independent classes. The former are lost by direct immunological assault in GVH and both types are destroyed by adrenal hyperactivity secondary to illness produced during GVH.

The non-thymectomized, adrenalectomized animals became lymphopenic because the predominant circulating lymphocytes were of the thymus-dependent type susceptible, we contend, to the immunological assault probably taking place in thymus-dependent regions of the peripheral lymphoid organs in the GVH reaction. However, since the thymus-independent lymphocytes are spared the immunological assault one would expect the degree of lymphopenia to be less severe. Because both types of lymphocytes were spared the cytolytic action of the adrenal secretions one would expect recovery to be more rapid as it was.

Thymectomized-adrenalectomized mice did not develop lymphopenia because a) the number of thymus-dependent lymphocytes had been decreased as a result of thymectomy, and the proportion of thymus-independent lymphocytes had increased so that they were the predominant lymphocytes present in the circulation and, b) adrenalectomy had eliminated the lymphocyte destruction on both cell systems ordinarily mediated by the adrenal glands during GVH.

Summary. Graft-vs.-host (GVH) reaction was induced in (C3H × C57Bl/1)F1 hybrid mice by intravenous injection of spleen-cells from adult C3H strain donors. The lymphocyte counts of recipients that had been thymectomized and adrenalectomized did not deviate significantly from those of uninjected control animals. Thymectomy or adrenalectomy alone did not prevent the development of lymphopenia but lymphopenia during GVH was of shorter duration in adrenalectomized than intact mice. We propose that the immunological assault in GVH is directed largely against thymus-dependent lymphoid elements and that the thymus-independent lymphocytes, not subject to the primary assault as well as thymus dependent lymphocytes may be destroyed indirectly in GVH by increased adrenal secretions.

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