# Thymic Involution in Murine Graft-Versus-Host Reaction

## *Epithelial Injury Mimicking Human Thymic Dysplasia*

Thomas A. Seemayer, MD, Wayne S. Lapp, PhD, and Robert P. Bolande, MD

**Mild, moderate, and severe graft-versus-host (GVH) reactions were induced in four series of experiments in 71 CBA × A and C57BL/6 × A $F_1$ hybrid mice. At regular intervals post-GVH reaction induction (Days 4–42), the animals were sacrificed, autopsied, and histologically studied. Visceral alterations of GVH reaction were recorded in the spleen, lymph nodes, liver, kidney, gut, and thymus. A spectrum of thymic changes was documented, ranging from obliteration of a definable cortex and medulla with loss of Hassall's corpuscles to marked involution with complete disappearance of the gland. Ultrastructural studies revealed damage to both lymphocytes and epithelial cells along with lymphocyte emperipolesis of epithelial cells, lymphocytolysis within epithelial cells, and accumulation of numerous autophagic vacuoles containing fragments of cellular debris within epithelial cells and histiocytes. The resemblance of these alterations to human thymic dysplasia as observed in primary immunodeficient conditions was striking. The theoretical implications of these studies for the pathogenesis of human congenital immunodeficiency states are considered. (Am J Pathol 88:119–134, 1977)**

A HOST OF PRIMARY CONGENITAL immunodeficiency states in man have been defined that display variable abnormalities in both cellular and humoral immunity.[1] The salient, indeed pathognomonic, feature of such conditions is the so-called congenitally *dysplastic thymus*. The dysplastic thymus is tiny, depleted of lymphocytes, shows altered corticomedullary demarcation, and above all, is devoid of Hassall's corpuscles. This thymic change is believed to be proximate to the T and B cell abnormalities constituting the various immunodeficiency states. The dysplastic thymus presumably results from an intrauterine failure of embryonic maturation or precocious involution. In the severe combined immunologic deficiency syndrome (SCID), at least nine different patterns of thymic dysplasia have been identified.[2] The manner in which these various types of thymic disease are initiated and evolve is unknown.

From our pathologic observations of thymuses in congenital immunodeficiency states and infantile graft-versus-host (GVH) disease, it became

From the Department of Pathology, The Montreal Children's Hospital and the Department of Physiology, McGill University Faculty of Medicine, Montreal, Québec, Canada.

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Address reprint requests to Dr. Thomas A. Seemayer, Department of Pathology, The Montreal Children's Hospital, 2300 Tupper Street, Montreal, Québec, Canada H3H 1P3.

apparent to us that the latter could induce a type of thymic involution barely distinguishable from some of the former. In these experiments, GVH reactions were induced in mice, mainly to study thymic reactivity under these conditions. The purpose was to learn if GVH reactions might play a role in the development of thymic dysplasia and thus contribute to our understanding of the pathogenesis of thymic-dependent immune deficiency states in man.

## Materials and Methods

Graft-versus-host reactions were induced in four groups of mice by the intravenous injection of $75 \times 10^6$ parental lymphoid cells (pooled spleen and lymph nodes) into 71 hybrid mice as previously described.[3] The mice ranged in age from 3 to 6 months. All four groups were maintained on an identical diet and in similar surroundings for the duration of each experiment. A mild to moderate (chronic) GVH reaction was induced in two groups (1 and 2) and a severe (acute) GVH reaction in the other two (Groups 3 and 4). In the former two groups, the reaction was produced with A($H\text{-}2^a$) lymphoid cells injected into CBA($H\text{-}2^k$) × A($H\text{-}2^a$) $F_1$ hybrids. The mild to moderate GVH reaction in this group is due to the fact that $H\text{-}2^k$ and $H\text{-}2^a$ have identical *K* and *I* regions but differ at the *D* region of the *H-2* complex. One week later, representative animals from each group were inoculated with sheep erythrocytes. Four days later their spleens were assayed for direct plaque-forming cells to sheep erythrocytes. At regular intervals following induction of the GVH reaction, the animals were sacrificed by cervical dislocation and autopsied (Table 1). The varying time intervals between the groups were necessitated due to the differences in the intensity and lethal potentiality of the reaction.

The tissues were immediately fixed in 10% buffered formalin. Whenever the thymus was not grossly apparent, the tissues of the neck, superior and anterior mediastinum were excised *en bloc* and serially sectioned. All tissues were embedded in paraffin, sectioned and stained with hematoxylin and eosin. The thymus and lymph nodes were also stained with methyl green–pyronine. In Group 4, the thymus in each animal was also studied by electron microscopy. For the latter a small fresh portion of gland was minced into 1-mm fragments, fixed in chilled 3% glutaraldehyde in phosphate buffer, rinsed in buffer, postfixed in osmium tetroxide, dehydrated in graded alcohols, and embedded in Epon. From several blocks of each thymus, 1-$\mu$ sections were cut and stained with toluidine blue. From representative areas ultrathin sections were cut, stained with uranyl acetate and lead citrate, and examined with a transmission electron microscope.

Controls in all four groups consisted of $F_1$ hybrid litter mates. These animals received no lymphoid inoculum. Two animals were sacrificed at the beginning and 2 others at the termination of the experiment and studied in an identical manner.

## Results

In all four experimental groups, the direct plaque-forming cell response was reduced to near background levels, thereby confirming host immunologic suppression as shown previously.[4,5]

Controls in all groups maintained a similar thymic structure and size (Figure 1). The gland exhibited a lobular pattern, with sharp corticomedullary demarcation and modest number of Hassall's corpuscles (Figures 2 and 3). The cortex was composed principally of lymphoblasts and lymphocytes. Epithelial cells, predominantly medullary in location,

Table 1—The Experimental Groups

| Group | No. of controls | No. of experimental animals | Interval of sacrifice post-GVH reaction induction |
|---|---|---|---|
| Mild to moderate GVH reactions | | | |
| 1 | 3 | 11 | 8–36 |
| 2 | 4 | 23 | 7–42 |
| Severe GVH reactions | | | |
| 3 | 4 | 22 | 4–18* |
| 4 | 4 | 15 | 4–18 |

* Two animals died during the experiment.

formed a delicate arborescent cytoreticulum with elongated serpentine cytoplasmic processes which extended into the cortex and capsule and also maintained a close relationship with cortical and medullary vessels. Epithelial cells were characterized by their large size, elongated cell processes, pale nucleoplasm, prominent nucleoli, well-formed Golgi zones, modest numbers of mitochondria, tonofilaments, desmosomal complexes, and basal lamina. Occasional epithelial cells contained variably sized membrane-bounded cytoplasmic vacuoles most of which were electron lucent, although lipid and granular electron-dense material were observed in some (Figure 4). Hassall's corpuscles were composed of aggregates of normal and degenerated epithelial cells with abundant densely packed tonofilaments and fragments of cell debris (Figure 5). An occasional eosinophil was identified in the medulla. Complete autopsies revealed normal skin, mucous membranes, and viscera with no features to suggest a GVH reaction.

In the inoculated animals, histologic evidence of a GVH reaction was apparent in multiple sites, the intensity of reaction being generally equivalent to that observed in the thymus. Lesions were identified in the spleen, lymph nodes, liver, kidney, lung, gut, skin, and tongue; the most intense occurred in the spleen and lymph nodes. The early splenic changes consisted of enlargement with an increase of immunoblasts and megakaryocytes in the red pulp. As the reaction progressed, Malpighian corpuscles disappeared, followed by a gradual reduction in splenic size accompanied by hemorrhage, necrosis, and eventual fibrosis. The lymph nodes enlarged and their germinal centers progressively disappeared. The nodal structure was gradually replaced to a considerable extent by pyroninophilic immunoblasts and histiocytes. The liver alteration consisted of an infiltrate of lymphocytes in portal triads, principally around portal veins and bile ducts with invasion of bile duct epithelium. No consistent changes were observed within the hepatic lobule. Multifocal interstitial

lymphoid infiltrates were present in the juxtamedullary renal cortex and medulla, principally related to venules. Similar infiltrates were observed in the lung in relationship to veins and venules, particularly those in fibrovascular septae. The skin was mildly infiltrated with lymphocytes at the epidermal–dermal junction with occasional intraepithelial lymphoid invasion. The tongue and gut similarly contained intramucosal lymphoid infiltrates; in the latter site, focal areas of complete mucosal necrosis were noted.

A spectrum of thymic changes including alterations to the epithelial thymus was induced by the GVH reactions. In Group I (mild GVHR), the principal observation was invasion of Hassall's corpuscles by lymphocytes (Figure 6) (Table 2). In Group II (moderate GVHR), considerable damage was inflicted, with resultant effacement of normal architecture; marked

Table 2—Thymic Alterations in Graft-Versus-Host Reactions

| Days postinduction | No. of animals | Size of gland | Corticomedullary demarcation | No. of Hassall's corpuscles |
|---|---|---|---|---|
| Mild GVH Reaction (Group 1) | | | | |
| 8 | 3 | n 3 | n 3 | n 3 |
| 15 | 4 | n 4 | n 4 | n 4 |
| 16 | 4 | ↓ 4 | a 4 | n 4 |
| Moderate GVH Reaction (Group 2) | | | | |
| 7 | 4 | ↓ 1 | a 1 | ↓ 1 |
| | | n 3 | n 3 | n 3 |
| 14 | 4 | ↓ 4 | a 4 | none 4 |
| 21 | 3 | a 2 | — | — |
| | | ↓ 1 | a 1 | none 1 |
| 28 | 4 | a 1 | — | — |
| | | n 3 | n 3 | rare 2 |
| | | | | none 1 |
| Severe GVH Reaction (Groups 3 and 4) | | | | |
| 4 | 6 | ↓ 6 | n 6 | n 6 |
| 6 | 2 | ↓ 2 | n 2 | ↓ 1 |
| | | | | n 1 |
| 7 | 4 | ↓ 4 | a 2 | none 2 |
| | | | n 2 | n 2 |
| 8 | 2 | ↓ 1 | a 1 | none 1 |
| | | a 1 | — | — |
| 11 | 2 | ↓ 1 | n 1 | rare 1 |
| | | ↓ ↓ 1 | a 1 | none 1 |
| 12 | 2 | ↓ ↓ 2 | a 2 | none 2 |
| 14 | 7 | a 1 | — | — |
| | | ↓ ↓ 6 | n 1 | rare 1 |
| | | | a 5 | none 5 |
| 16 | 2 | ↓ ↓ 2 | a 2 | none 2 |
| 18 | 5 | ↓ ↓ 5 | a 5 | none 5 |

n = normal, ↓ = slight reduction in size, ↓ ↓ = marked reduction in size, a = absent.

reduction in size; and disappearance of a definable cortex, medulla, and Hassall's corpuscles maximized 21 days after induction (Figure 1). In 3 animals approximately midway into the experiment (Day 21 and 28) the thymus was not present. This thymic change was followed by partial restitution of structure during the 28- to 42-day postinduction period (Table 2). The two groups with severe GVHR (Groups III and IV) were characterized by rapid reduction of thymic size and lymphoid cell mass, and obliteration of architecture with loss of definable cortex, medulla, and Hassall's corpuscles (Figures 7 and 8). No recovery of structure was observed in the two latter groups. In 3 animals the thymus was not present despite *en bloc* excision and serial sectioning, suggesting that the reaction had produced total destruction of the gland (Table 2).

Fine structural studies documented striking changes in many but not all epithelial cells, including marked cytoplasmic vacuolization, with the formation of numerous autophagic vacuoles and cytolysosomes containing membranous and granular subcellular fragments (Figure 9). Lymphocyte emperipolesis of epithelial cells was occasionally observed (Figure 10), as was extensive intraepithelial lymphocytolysis and lymphocytorrhexis (Figure 11) with the accumulation of electron-dense granular cellular debris within epithelial cells. Large (9 to 10 $\mu$) lymphocytes with pale euchromatin, prominent nucleoli, and abundant cytoplasm replete with polyribosomes were commonly juxtaposed to damaged epithelial cells (Figure 12). These contrasted with the more numerous small lymphocytes (5 to 6 $\mu$) with condensed heterochromatin and scant cytoplasm. The former possibly represent activated aggressor lymphocytes. Lymphocytes remote from altered epithelial cells also underwent lymphocytolysis and lymphocytorrhexis with incorporation of their cellular components within histiocytes. As the intensity and the duration of the reaction progressed, eosinophils and histiocytes—the latter replete in cytolysosomes, autophagic vacuoles, and fragments of cellular debris—became more numerous, particularly in proximity to altered epithelial cells (Figure 13).

## Discussion

The GVH reaction, a T cell–mediated reaction directed against constituents of the major histocompatibility complex, is dependent upon three principal factors: a) the graft must be immunologically competent, b) the host must be genetically different from the donor, and c) the host must be immunologically incapable of rejecting the graft. The intensity of the reaction is related to the quantity and route of administration of immunologically competent cells, the degree of host–graft antigenic disparity, and the extent of host cellular immunologic deficiency. The latter

requisite may be met following irradiation, and with immunosuppressive therapy, viral infection, or malignancy. The fetus and children with hereditary primary cell-mediated immunodeficiency are equally vulnerable. In the laboratory animal, cellular immunodeficiency may be achieved following neonatal thymectomy, the administration of antilymphocyte serum, near-lethal irradiation, or by utilization of the parent to $F_1$ hybrid model.[6] The latter represents the classic experimental method and was the one employed in this study. By employing inbred mice strains in which a difference exists at the major histocompatibility *H-2* locus, the subsequent injection of parental lymphoid cells into the $F_1$ hybrid induces a GVH reaction. The intensity of reaction can be modulated by strain selection, quantity of graft inoculum, and route of administration of the inoculum.

Earlier studies have shown that the GVH reaction, induced across a major histocompatibility barrier, suppressed both cell-mediated and humoral immune responses.[4,5,7] It was suggested that a thymic deficiency was responsible for immunosuppression since the transplantation of a whole thymus into animals experiencing a GVH reaction dramatically increased the intensity of the GVH reaction as measured by animal survival time, weight loss, and the ratio of animals suffering from acute and chronic GVH disease.[8] There is also sufficient evidence to suggest that suppression of the humoral immune response is due, at least in part, to suppressed T-cell helper function [3,5,9,10] without a concomitant deficiency of T cells [5] and is mediated by macrophages.[11] Thus there is ample evidence to suggest that a T-cell defect may be responsible for GVH-induced suppression of both cell-mediated and humoral immune responses; whether or not the same defect is responsible for suppression of both types of responses awaits confirmation.

The essential stimulatory target for donor T cells is the host lymphocyte. This is probably related to the high concentration of *H-2* antigens on lymphoid cells, particularly those in the spleen and lymph nodes. The epithelial cells of the thymus also contain *H-2* antigens and, theoretically, should also be vulnerable to immunologic attack. These antigenic differences activate donor T cells to become sensitized effector T cells; this is manifested in a cell-mediated immune reaction resulting in damage to those organs in which the reaction occurs. The extent of damage is not restricted to lymphoid tissue such as spleen, lymph nodes, and bone marrow alone, but also affects epithelial tissue, as reflected by the distribution of lesions in skin, mucous membrane, gut, liver, and kidney.[12]

In human GVH reactions the thymus has been thought to be relatively unaffected, as have the sex organs, central nervous system, endocrine

organs, lung, stomach, and heart.[13] Thymic atrophy or involution has been described in experimental murine GVH reactions and attributed to an hormone-mediated phenomenon dependent upon the integrity of the pituitary–adrenal axis rather than immunologic mechanisms. This atrophy has been histologically characterized as a disorganization of thymic morphology with destruction and depletion of thymocytes, and depletion of cortical lymphocytes. The thymic epithelium was not mentioned in these studies, however.[14]

The fine structural changes in the thymus have been described in experimental runt disease in Sprague-Dawley rats and C57BL/6 mice. The salient alterations included depletion of small lymphocytes with accompanying nuclear and cytoplasmic damage leading to cytolysis and cell death. No significant injury was described in epithelial cells.[15]

The GVH reaction in skin and mucous membrane has been described at the ultrastructural level in the rhesus monkey.[16] Epithelial cells sustain a progressive loss of desmosomes initially leading to acantholysis. Cytoplasmic changes in epithelial cells adjacent to aggressor lymphocytes include vesiculation of endoplasmic reticulum, alterations in mitochondria, and formation of autophagic vacuoles containing granular material and cellular debris. The most striking change follows lymphocyte envelopment of epithelial cells, with the production of a condensed retracted necrotic cell. While most of the structural alterations affected epithelial cells, some lymphocytes sustained degenerative alterations leading to cell fragmentation and necrosis. Unfortunately, the thymus was not examined.

This study has documented injury to both thymic epithelium and lymphoid components in murine GVH reactions. The damage was of such intensity that we were unable to find the gland in 6 animals despite *en bloc* excision and serial sectioning. From this study, one cannot state if the thymic alterations are pathognomic for a GVH reaction. Indeed, some of the changes resemble those described in the thymus following administration of corticosteroids and after irradiation. Yet although marked thymic involution occurs, the effect of corticosteroids and irradiation is principally on thymic lymphocytes with sparing of Hassall's corpuscles. Moreover, emperipolesis is not described.[17-21] Similarly, over the years we have examined, at autopsy, the thymuses of children treated with corticosteroids and/or irradiation for a variety of conditions. The Hassall's corpuscles in these glands, rather than being absent, are rather prominent. The mechanism by which the thymic injury is effected in a GVH reaction is yet to be determined. Possibilities would conceivably include a relationship with the adrenal cortical–pituitary axis, the release of endotoxins, the

activation of a virus in the thymus, or direct injury by aggressor lymphocytes. Further investigation has been initiated to explore these possibilities.

These observations offer a plausible explanation for the described association of thymic dysplasia, lymphoid chimerism, and primary immune deficiency in children.[22-24] The data provide a mechanism relating thymic dysplasia and immune deficiency to an intrauterine graft of maternal lymphocytes [25,26] or to intrauterine or postnatal exchange blood transfusions.[27,28] Most primary immune deficiencies follow genetic inheritance patterns, and there appears to be a heightened incidence with inbreeding and consanguinity.[29] Thus, a genetically determined factor may facilitate placental transfer and/or activation of immunocompetent and reactive maternal cells in the fetus. If, through such mechanism(s) maternal lymphocytes are rendered capable of initiating a GVH reaction, a possible effect on the developing fetus would be thymic injury with the production of congenital immunodeficiency.

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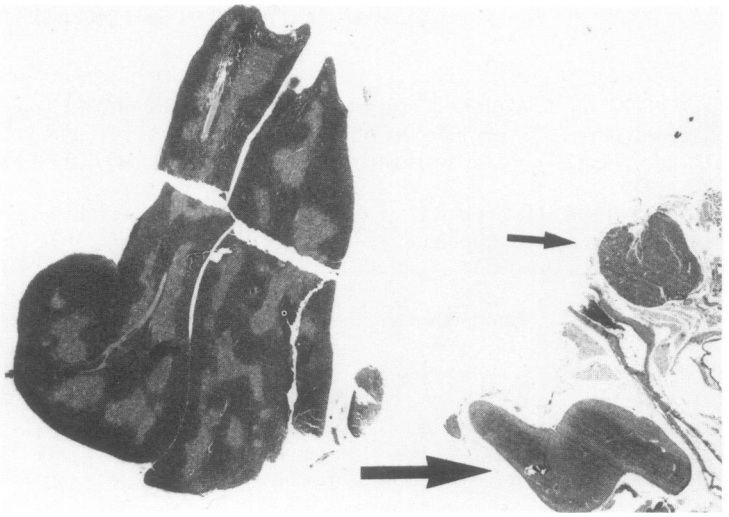

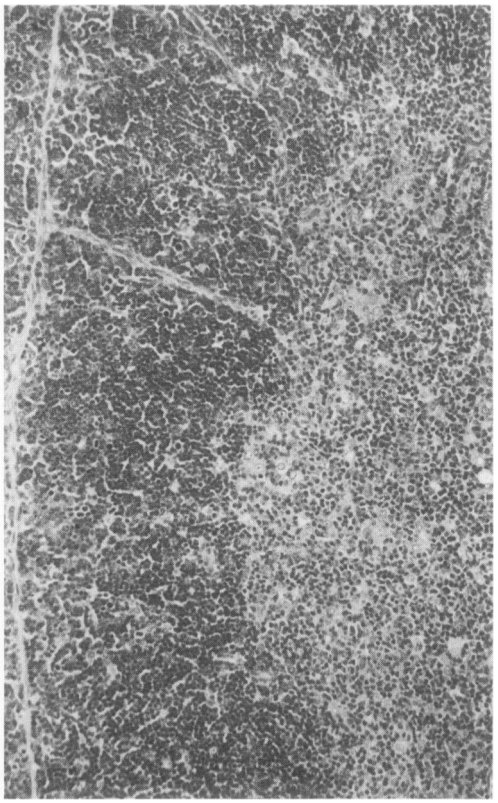

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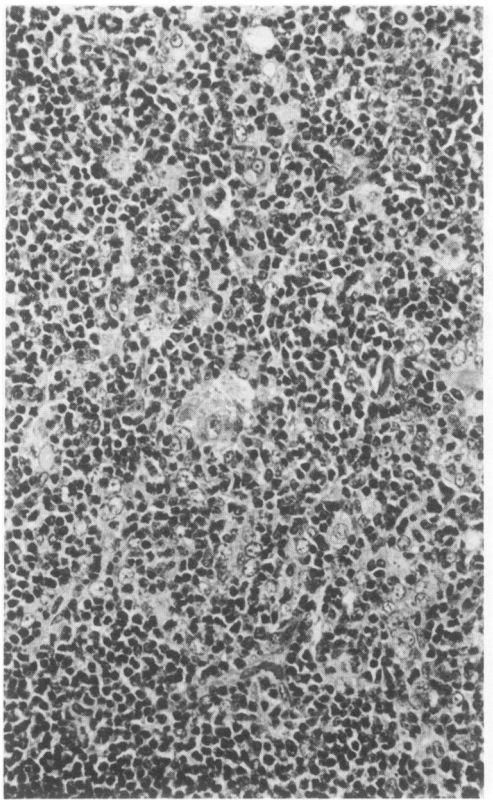

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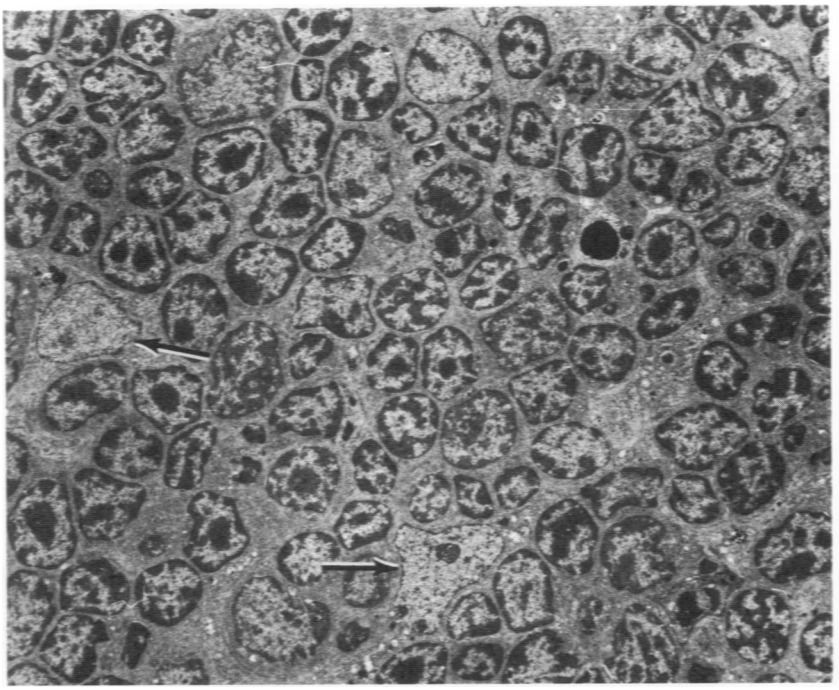
**Figure 1**—Montage demonstrating marked thymic involution in GVH reaction. Control thymus is at left; thymus (*large arrow*) and lymph node (*small arrow*) from Group 2, Day 14 are at right. (H&E, × 9) **Figure 2**—Photomicrograph of control thymus illustrating distinct corticomedullary demarcation (H&E, × 140). **Figure 3**—Photomicrograph of medulla from control thymus containing Hassall's corpuscles (H&E, × 250).



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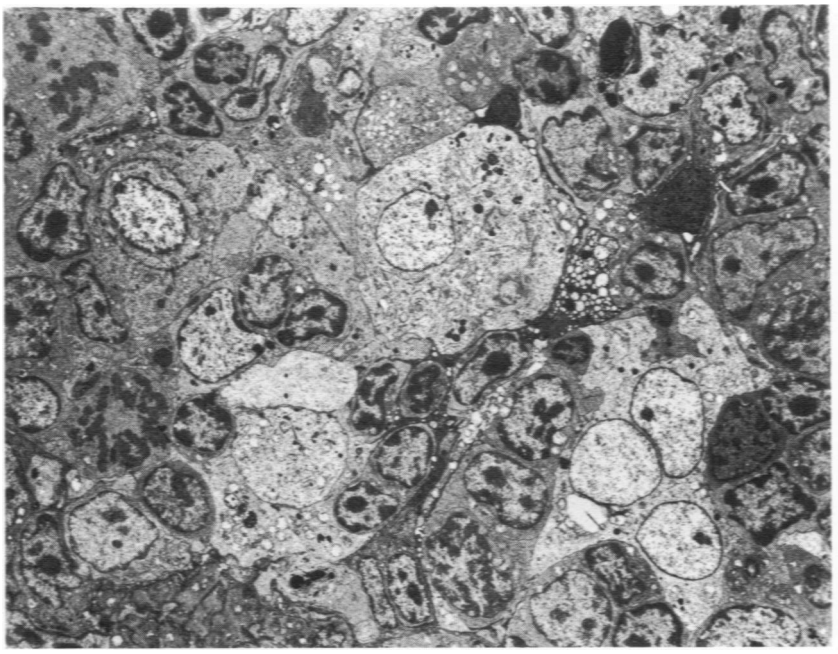

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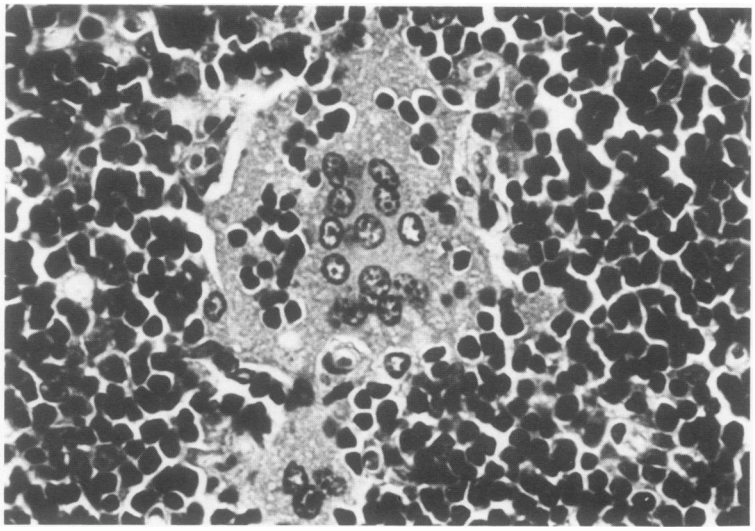
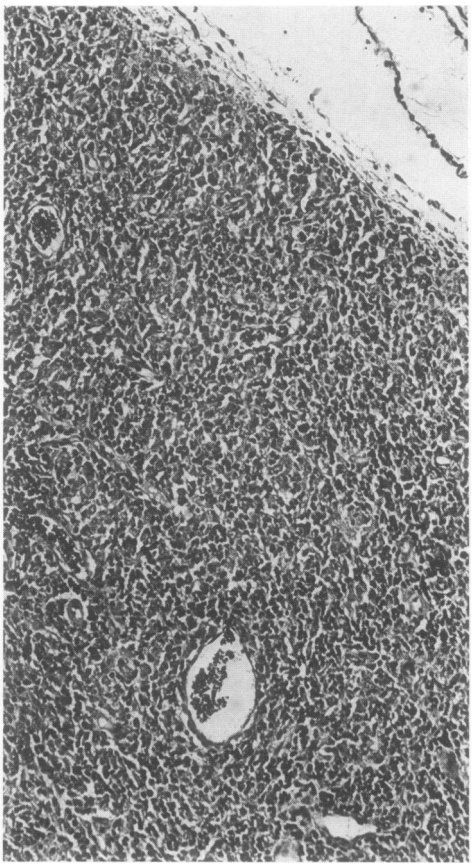
**Figure 4**—Electron micrograph of thymic cortex from control showing predominantly lymphocytes with interspersed epithelial cells (*arrows*) (× 1950). **Figure 5**—Electron micrograph of medulla from control thymus illustrating aggregate of epithelial cells representing an Hassall corpuscle (× 1950).





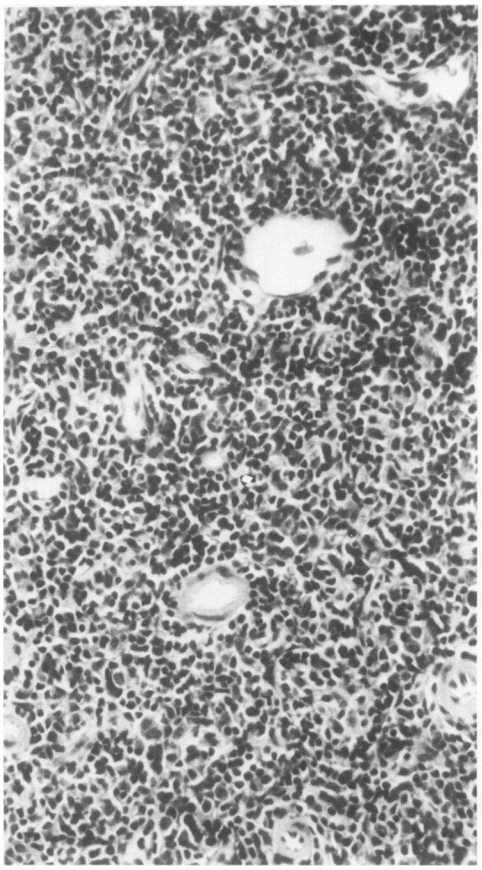


**Figure 6**—Photomicrograph of thymus in Group 1 animal (mild GVH reaction) depicting lymphocytes within Hassall corpuscle (H&E, × 560). **Figure 7**—Photomicrograph of thymus in Group 3 animal (severe GVH reaction) illustrating absence of corticomedullary demarcation (H&E, × 140). **Figure 8**—Photomicrograph of thymus in Group 4 animal (severe GVH reaction) depicting prominent vascularity with no recognizable Hassall's corpuscles (H&E, × 250).

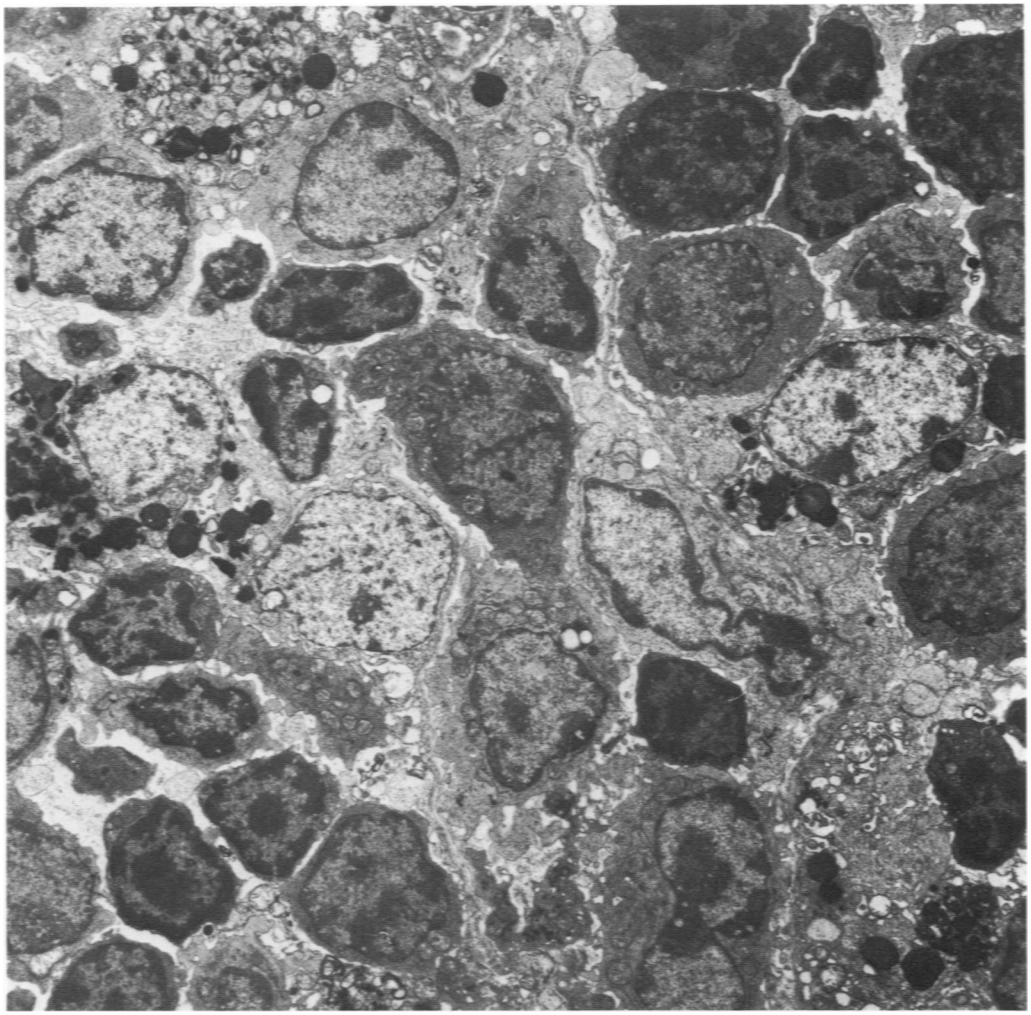


**Figure 9**—Electron micrograph (Day 8 of experiment) showing accumulation of electron-dense debris within epithelial cells (× 3800).

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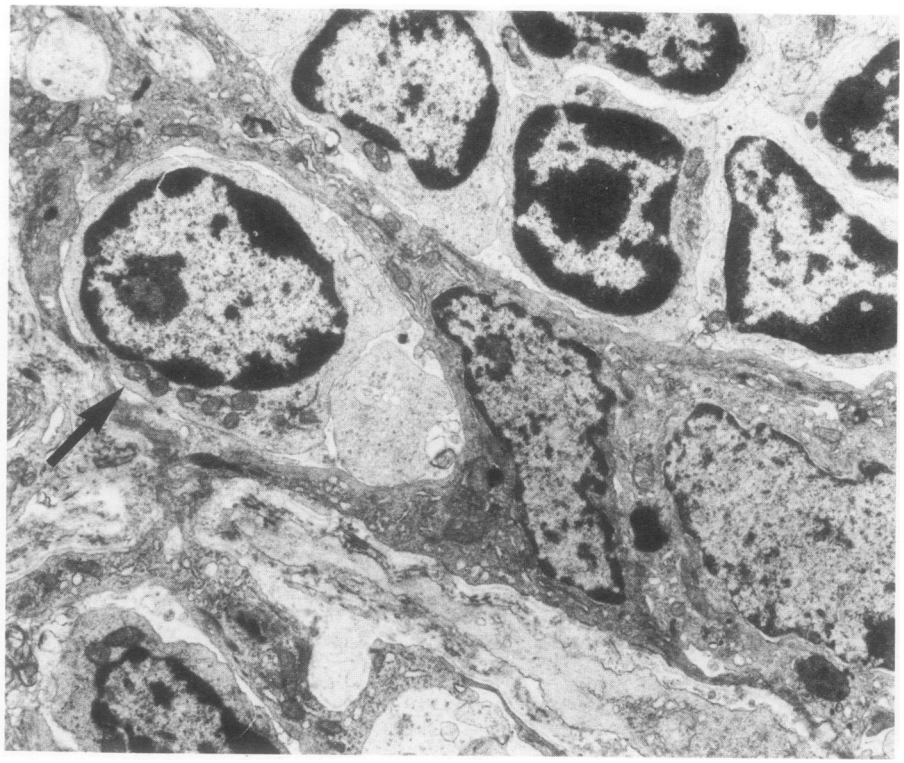

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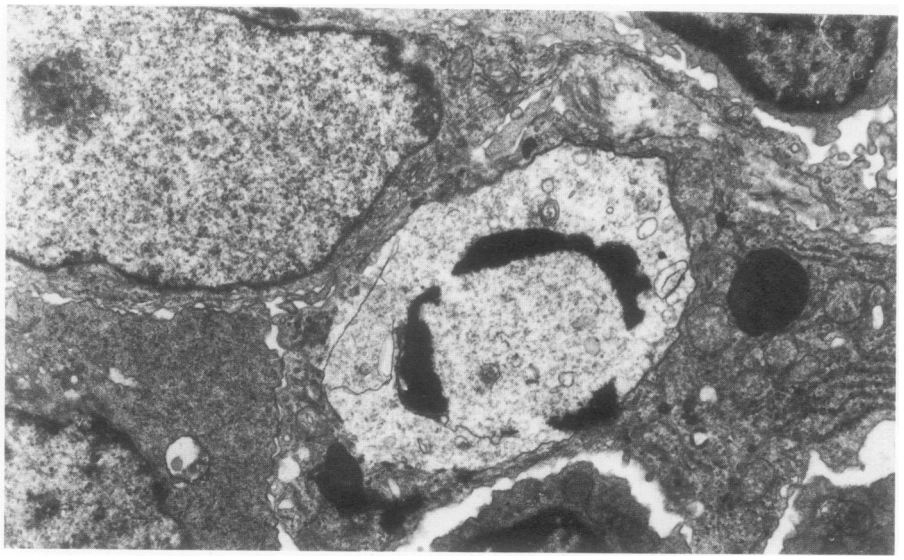
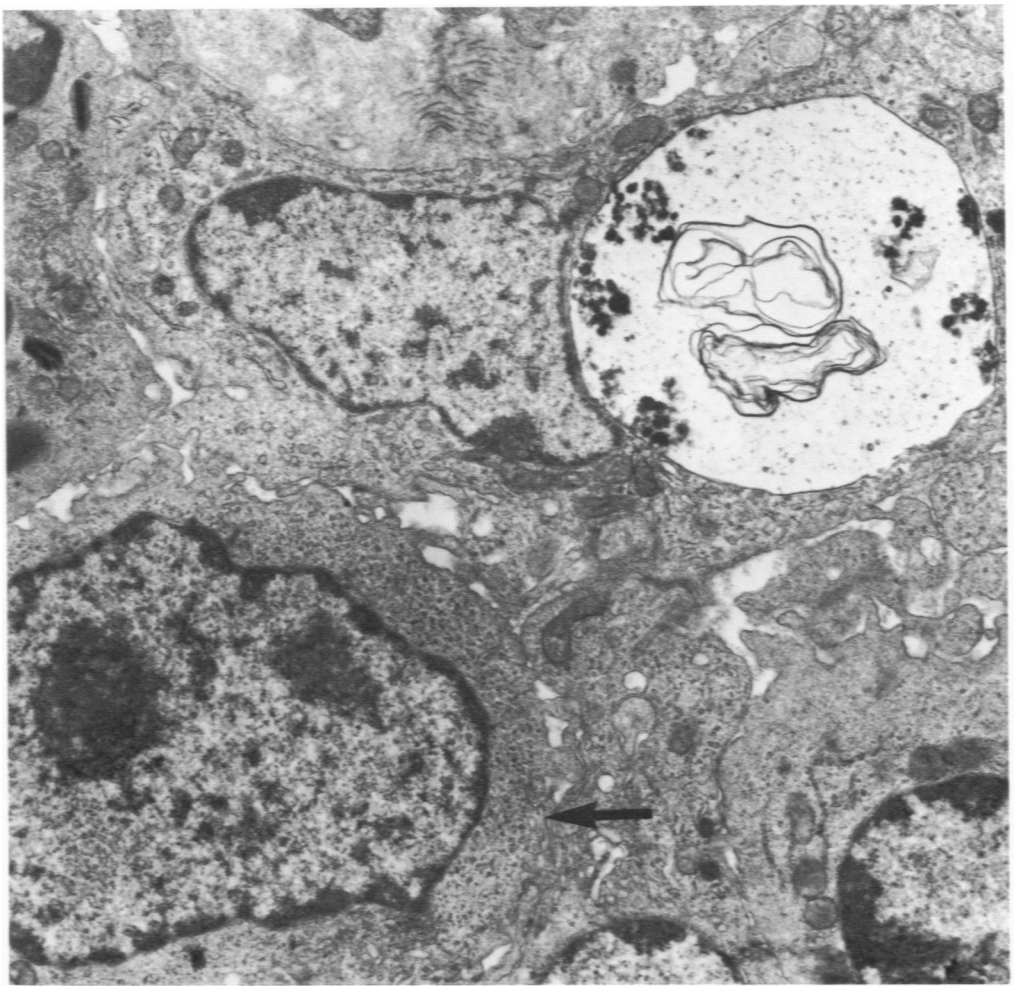


**Figure 10**—Electron micrograph (Day 8 of experiment) demonstrating lymphocyte (*arrow*) emperipolesis of epithelial cell (× 7500). **Figure 11**—Electron micrograph (experimental (Day 11 of experiment) illustrating chromatolysis and cell death within intact epithelial cell (× 9800).



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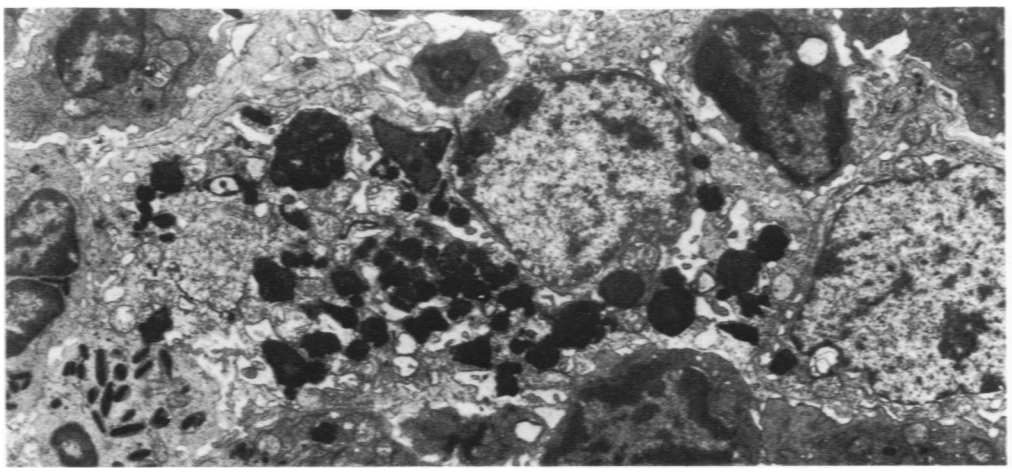

13

**Figure 12**—Electron micrograph (Day 11 of experiment) depicting altered epithelial cell with vacuole containing cellular debris adjacent to which is a lymphoblast (*arrow*) (× 11,250). **Figure 13**—Electron micrograph (Day 8 of experiment) illustrating damaged epithelial cell with adjacent eosinophils (*left*) (× 5700).

[*End of Article*]