A Survey of the Thymus and Its Relation to Lymphocytes and Immune Reactions

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Recent extirpation studies from several laboratories have indicated that the thymus may play a major role in immune reactions. In rabbits thymectomized at birth, there is suggestive evidence that humoral antibody production is impaired.1 Rats thymectomized shortly after birth have a diminished capacity to make antibody against and to develop Arthus sensitivity to bovine serum albumin (BSA).2 Thymectomy of adult rabbits, however, appears not to affect ability to make antibody.3-5 Guinea pigs thymectomized as adults have been reported to show a slight impairment of antibody formation.6

The response of the thymectomized animal is also impaired in a variety of delayed hypersensitive reactions. Miller,7 Martinez et al.8 and we9,10 have shown that skin homograft rejection is markedly delayed in animals thymectomized shortly after birth. Rats thymectomized within a week of birth have a compromised capacity to develop both tuberculin sensitivity and delayed reactivity against BSA—a pure protein antigen.2,11 The thymectomized rat is incapable of developing experimental allergic encephalomyelitis, an autoimmune disease thought to be mediated by delayed hypersensitivity.9-11 Adjuvant induced rat arthritis, however, appears to be uninfluenced by thymectomy.11

The histologic concomitant of this gross immunologic incompetence is a specific depletion of small lymphocytes from blood, spleen and lymph nodes.7-9,11,12 In contrast, at least in the rat, germinal centers, plasma cells, and reticuloendothelial elements (blood monocytes, Kupffer cells, microglia of nervous tissue, reticulum cells of splenic red pulp) are unaltered by thymectomy, and plasma protein values, including γ-globulin, are normal by paper electrophoresis.2,12 Miller has shown that replacement with intact viable thymus restores homograft reactive capacity in the mouse.7 Injection of thymic homogenates, in contrast, does not restore immunologic competence to the thymectomized rat.2

Thymectomy of older animals has generally failed to influence antibody production4-6 or delayed reactions5,7,8,11 although rats thymectomized as late as 3 weeks after birth have shown a decreased delayed reactive capacity against protein antigens.2 It is possible that early thymectomy is necessary to produce immunologic incompetence. This situation is perhaps analogous to that in chickens undergoing surgical removal of the bursa of Fabricius. The bursa, a lymphoid structure strikingly similar to the thymus, arises as an out-

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pouching of cloacal entoderm. Bursectomy of newly hatched chickens results in a marked inhibition of antibody forming capacity, but fails to influence homograft rejection. Bursectomy of birds at ages greater than 5 weeks does not in any way alter antibody forming capacity even if several months are permitted to elapse between resection and sensitization. Thus far, studies of immune responses after thymic extirpation in older animals have all involved immunologic manipulation within a relatively short interval after operation. Studies several weeks or months after thymectomy have not thus far been reported.

The present survey is an attempt to correlate and interpret general information about the thymus in the light of the recent immunologic developments outlined above. Embryologically the thymus is a bilobed structure derived from the entoderm of the third pharyngeal pouch on each side. In the rat it lies entirely within the thorax. In man it is predominantly intrathoracic but may extend upwards into the lower neck. In the guinea pig it is wholly cervical. Ectopic nodules of thymic tissue may be found in the neighborhood of the thyroid, often closely associated with the parathyroid glands, in man. The same is true of the rat, though in this species the incidence of ectopic thymus is low. In the guinea pig, accessory thymus in the region of the parathyroid is the rule, and in hoofed animals there is, in addition to a bilobed thoracic thymus, a separate distinct cervical thymus.

Each lobe of the thymus consists of numerous lobules held together by fibrous tissue. Individual lobules contain several follicles, and each follicle has cortical and medullary portions. The cortex is composed predominantly of lymphocytes, with some larger cells, thought to be lymphocyte precursors. The origin of these lymphocytes has long been in dispute. Some have held that they and their precursors migrate into the thymic anlage from the surrounding mesenchyme; others have held that they are derived from entodermal cells of the thymic anlage itself. Auerbach has recently—at least for the mouse—laid this controversy to rest by showing, in a series of carefully controlled experiments, that the primitive entodermal cells of 12-day mouse embryo thymus differentiate into lymphocytes upon transplantation into the anterior chamber of the eye in adult mice of the same strain and will even grow and differentiate into lymphocytes under favorable conditions in vitro. Mesenchyme is necessary for this differentiation but acts as the source of an inducing agent rather than as cell donor. The regeneration of mouse thymus after depletion of its lymphocytes by irradiation can be explained entirely by rapid mitosis of residual cells in the thymus itself. Transfusion of isologous spleen or bone marrow does not accelerate thymic regeneration, nor do isologous spleen and bone marrow cells migrate to the thymus. There is, however, evidence that a bone marrow factor, possibly a maturation factor, may accelerate the regeneration of thymus following irradiation.

In man, the absolute mass of the thymus increases throughout childhood; it is maximal at or about puberty, and thereafter slowly decreases. Involution of the thymus continues throughout adult life until it becomes a mere remnant in old age, though Hammar has maintained that it may persist and
function even in advanced life. In rats, the thymus is of considerable size throughout the adult reproductive period, but its mass tends to diminish in extreme old age. Histologically, the thymus of the newborn presents the normal adult architecture and appears fully functional at a time when other lymphatic tissues are very incompletely developed. Similarly in germ-free animals, in which the lymph nodes remain rudimentary, the thymus maintains its activity. While in both neonatal and adult germ-free animals an extrinsic antigenic stimulus is required to induce normal development of other lymphatic organs, the stimulus for development and function of the thymus would seem to be intrinsic.

The size and activity of the thymus is influenced throughout life by complex interacting endocrine factors. Castrates retain large thymuses throughout adulthood. Both testosterone and estrogens are thymolytic; in females, pregnancy hastens thymic involution. Thymectomy does not, however, influence sexual maturation or function. The thymus becomes hyperplastic after adrenalectomy and in Addison’s disease (see ref. 36 for review). The elevated blood lymphocyte count which follows adrenalectomy is not seen in thymectomized animals. Adreno-corticosteroids cause a marked acute involution of the thymus as part of the “stress-reaction.” Hyperthyroidism, as is well known, is associated with enlargement of the thymus while thyroidectomy hastens its involution. Interestingly, adrenalectomy fails to produce thymic hyperplasia in thyroidectomized rabbits, suggesting that an intact thyroid may be essential for thymic regeneration.

Thymectomy has been said to cause thyroid hyperplasia and hypophysectomy to cause shrinkage of the thymus, though how these effects are mediated is unclear. It has been claimed that the thymus inactivates thyroid-stimulating hormone.

The early history of thymus extirpation experiments has been the subject of two comprehensive reviews. It has been clearly shown that thymectomy in early life does not, in animals which remain healthy, lead to any alteration in growth, weight gain or maturation. Thymectomized animals have, in many experiments, shown an undue susceptibility to infection and chronic wasting, so-called thymic cachexia. Perhaps the most striking fact established in earlier studies is that thymectomy in rats, mice, guinea pigs, rabbits, and man, at any age from birth through adulthood, is followed by a fall in the peripheral blood lymphocyte count. This fall is slowly progressive over several weeks and the count remains low for many months. The lymphocytic response to trauma is abolished by thymectomy. The output of lymphocytes from the thoracic duct is markedly depressed by thymectomy despite the fact that the thymus does not itself contribute any appreciable number of cells to the thoracic duct lymph. In addition to the marked decrease in small lymphocytes of the blood, the thymectomized animal shows clear evidence of depletion of lymphocytes in the spleen and lymph nodes. These organs are actually smaller in the thymectomized than in the normal animal. Surgical excision of a major portion of the lymphatic tissue is followed by compensatory hypertrophy of
the remainder. However, if the thymus is taken at the time of excision of other lymphatic tissue, compensatory hypertrophy fails to occur. Microscopic examination of spleen and lymph nodes in rats thymectomized at birth reveals an almost total absence of small lymphocytes; yet plasma cells are present in normal complement and germinal centers are intact. Miller, however, has reported a failure of germinal center development in the mouse thymectomized at birth. Since germinal centers and plasma cells are absent from the lymph nodes of the newborn animal and thymectomy, at least in the rat, in no way alters their subsequent development, the influence of the thymus on these elements is conjectural.

There are at least two, readily apparent, plausible explanations for the lymphocyte depletion of thymectomized animals. The first, and inherently the simpler, is that the thymus is a major source organ for lymphocytes and that lymphocytes manufactured in the thymus migrate, via lymphatic channels which do not open into the thoracic duct via thymic blood vessels, to the systemic circulation, thence to seed the other lymphatic tissues. Andreason and Ottesen, utilizing P, measured the relative rates of DNA synthesis in various lymphatic tissues of the rat. The highest rate of lymphocyte production at all ages was found in the thymus; compared to thymus, other lymphoid organs appeared to possess only a slight lymphocytopoietic function. Kindred and Yoffey postulated on the basis of mitotic counts that the thymus acts as a source of lymphocytes for other tissues. Sainte-Marie and Leblond, in a study of lymphocyte formation in the rat thymus, found a very high mitotic frequency in the thymic cortex. In the thymic medulla, they noted cells of the lymphocyte series with irregular configurations suggestive of ameboid motion and observed diapedesis of small lymphocytes into the endothelial-lined, perivascular, presumably efferent, lymphatic channels originally described by Hammar. Smith has seen similar lymphatic vessels full of lymphocytes in the mouse thymus. Sainte-Marie and Leblond suggested that the thymic medulla might contain a factor, possibly secreted by reticular cells, responsible for attracting lymphocytes from cortex to medulla and perhaps responsible for their diapedesis across endothelium as well.

While it has been assumed in the past that germinal centers of the spleen and lymph nodes are the major source of small lymphocytes, the data given above point to the thymus as a major source of these cells. It is clearly established that the lymphocyte circulates through the lymph nodes, and the anatomic pathway for this circulation has been demonstrated. The localization of transfused small lymphocytes in splenic white pulp has also been demonstrated by tracer methods. These observations are consistent with the view that the small lymphocytes of these two lymphoid tissues come via the circulation from the thymus.

The second ready explanation for the low lymphocyte count following thymectomy is that the thymus may act as an endocrine organ and secrete a hormone which stimulates lymphopoiesis. Serial injection of thymic extracts has been claimed to cause lymphocytosis in intact animals, but this could well be an immune lymphocytic response to antigen contained in the
extract. Recently Metcalf has described a "lymphocytosis stimulating factor," extractable from the medulla of normal human or mouse thymus and from no other organ, which, when injected intracerebrally into newborn mice, causes a blood lymphocytosis maximal at about 6 days. This factor will maintain the lymphocyte levels of thymectomized adult mice. High leukemia strain mice, in whom the lymphocyte count is high from birth, do not respond to it. The titer of lymphocytosis stimulating factor in the thymus is low at birth but rapidly increases to high levels. It can be found in the involuted thymus of elderly humans, a finding which suggests that the functionally active cells may be Hassall's corpuscle cells. It is present in the serum of young adult high leukemia mice but can no longer be found after thymectomy. Tissue cultures of thymic fragments produce it; adrenal hormones suppress its production. In apparent contradiction to these observations, "lymphopoiesis," as measured by pickup during DNA synthesis or by mitotic counts (but see ref. 45), is not altered in the lymph nodes following thymectomy. The failure of thymic homogenates to restore immunologic competence to the thymectomized rat would argue against any exclusively hormonal role for the thymus. On the other hand, the restoration of homograft reactivity to thymectomized mice by transplantation of intact viable thymus, suggesting as it does that the thymus is a direct source organ of small lymphocytes, does not exclude an ancillary hormonal function.

Lymphocyte depletion after thymectomy, even early in life, is selective. The peripheral blood is never wholly depleted, and bone marrow and Peyer's patch lymphocytes are unaltered. The residual small lymphocytes after thymectomy may be derived from alternative source organs and may represent a second population of cells, not necessarily concerned with immune functions. Turnover studies indicate that, in the rat and in man, there are two discrete blood lymphocyte populations with different life spans. Labeled lymphocytes of thymic origin, on transfusion, preferentially localize to spleen and liver, while labeled lymphocytes of lymph node origin localize to bone marrow and liver.

The importance of the thymus for lymphocyte production is emphasized by studies of lymphatic leukemias in mice. Thymectomy markedly lowers the incidence of spontaneous leukemia in high leukemia strain mice (see ref. 34 for review). It also reduces the incidence of radiation-induced lymphomas in mice as well as the incidence of methylcholanthrene-induced lymphomas, but not carcinomas. Thymic grafting, in isologous combinations, restores the potentiality for lymphoma development. Low leukemia strain mice, made tolerant to and grafted with high leukemia strain thymus, subsequently develop lymphomas, some of which can be shown to be of host origin. Conversely, thymectomized high leukemia strain mice, tolerant to and grafted with low leukemia strain thymus, do not develop lymphoma. Injection of inducing virus fails to produce leukemia in thymectomized mice. The leukemogenic agent, of mouse leukemia, can be serially passed through thymectomized mice which never themselves develop leukemia.

The immunologic competence of thymic lymphocytes, as such, is conjectural.
The thymus ordinarily contains few plasma cells and germinal centers are lacking. The thymus is not a significant producer of antibody, either in vivo, on passive transfer, or in vitro. Antibody has been said to be produced by intraocular transplants of mouse thymus in irradiated recipients, even when the transplanted fragments contained neither mature lymphocytes nor plasma cells. Mouse thymus and “attached lymph nodes” have been used to transfer tuberculin sensitivity passively in the mouse, but whether this depended upon the thymus cells or upon the lymph node cells is unclear. Skog claimed success in passive transfer of tuberculin sensitivity with thymus cells, but his recipient animals gave reactions so small as to leave the matter in considerable doubt. Injection of adult mouse thymus cells into newborn mouse recipients can cause the runting syndrome, a graft versus host reaction, albeit “rather fitfully”; but rat thymus cells have a very feeble ability, even in high doses, to cause runt disease. Similarly, the production of secondary disease in lethally irradiated mice required 30 times as many homologous thymus lymphocytes as homologous lymph node cells. To prevent the therapeutic action of homologous bone marrow in lethally irradiated mice required two to four times as many isologous thymus lymphocytes as isologous lymph node cells.

Recently, Marshall and White were able to induce formation of germinal centers and plasma cells within guinea pig thymus by direct local injection of protein or bacterial antigens. They suggested that the failure of the thymus to respond to antigen given by conventional routes depends upon its sequestration and postulated the existence of a blood thymus barrier. Germinal centers, as is well known, are commonly seen in thymuses removed from patients with myasthenia gravis. Whether they are present in other disease processes has been inadequately studied. Burnet and Holmes have found them in association with a spontaneously developing hemolytic anemia in the thymuses of mice of the NZB/BL strain. It is not clear from these observations that the thymus itself responds to antigenic stimulation. Blau injected protein antigen in mycobacterial adjuvant directly into the rat thymus. Neither Arthus nor delayed hypersensitivity developed, whereas the same dose of antigen injected directly into lymph node or foot pad uniformly produced both. However, plasma cells were present in the injected thymuses. Kuhlman transplanted testicular homografts directly into guinea pig thymus and found prolonged rather than shortened survival. O’Cara and Ards transplanted thymus fragments from newborn mice into spleens of adult mice of the same strain, and observed the transplants for up to 2 years. Despite their new, non-sequestered position, germinal centers did not develop in the transplanted thymuses. The mere presence of germinal centers or plasma cells within the thymus, however, does not prove their origin from thymic elements. Conceivably the precursor cells for these could originate without rather than within the thymus. From the above evidence one must conclude that the immunologic competence of thymic lymphocytes, at least while still resident in the thymus, remains in considerable doubt.

In the thymectomized rat there is an exact relationship between the degree
of small lymphocyte depletion and the extent of the immunologic deficit.\textsuperscript{11,12}
This is in accord with the already considerable evidence linking the lymphocyte to hypersensitivity (reviewed in ref. 102). The status of the thymectomized animal invites comparison with human diseases in which there is a loss of immunologic function. In the hypogammaglobulinemias,\textsuperscript{102} a failure to make antibody or to resist acute pyogenic infections is coupled with an absence of germinal centers and plasma cells from the lymphoid organs and of \(\gamma\)-globulin from the serum. Blood lymphocyte levels and delayed reactive capacity are normal, however. The thymectomized animal shows a closer resemblance to the less well studied human cases of lymphopenia which occur in association with hypogammaglobulinemia,\textsuperscript{104} and in which there is a loss of resistance to various viral, fungal, and chronic bacterial infections.

\textit{In summary}, the thymus appears to be a principal source of the small lymphocyte population of blood, spleen and lymph nodes. It may, in addition, produce hormonal factors affecting lymphocyte production. It plays a major role, early in life and perhaps later as well, in maintaining the immunologic integrity of the organism.

**SUMMARIO IN INTERLINGUA**

Recente studios de extirpation in varie laboratorios ha indicate que le thymo es de importantia major in le mechanismo del reactiones immunologic. Es presentate un revista del pertinente litteratura. A base de isto, insimul con constatationes per le autores, illo pare justificate asserer que le thymo es un fonte principal del population de micre lymphocytos del sanguine, del splen, e del nodos lymphatic. Il es possibile, in plus, que illo produce factores hormonal que affice le production de lymphocytos. Illo ha un rolo major—durante le prime partes del vita e forsan etiam plus tarde—in mantenere le integritate immunologic del organismo.

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