The Thymus and the Development of Immunologic Responsiveness

The thymus directs the maturation of immunologic capabilities by means of a humoral mechanism.

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An adequate defense system capable of dealing with foreign invaders is a requirement of the utmost importance for the survival of a species. Phagocytic and enzymatic mechanisms appear to be the only methods available to invertebrates for the destruction of pathogens (*1*). In addition to these methods, most vertebrates have developed an immunologic apparatus which enables them to produce specific reactions to limit the spread of pathogens or to prevent damage immediately in subsequent attacks. The reactions associated with this system are the production of specific antibodies or immunoglobulins, the development of delayed hypersensitivity (such as tuberculin sensitivity), and the rejection of grafts of tissues from foreign donors. Phylogenetic studies (*2*) have suggested that the evolution of such an immunologic apparatus depended upon the appearance of the thymus and organized lymphoid structures. Ontogenetic studies (*3*) have revealed that the timing of immunogenesis in the developing vertebrate roughly corresponds to the period at which lymphoid elements, notably lymphocytes, can first be identified. The thymus is the first organ in which such elements can be clearly recognized (*4*). It develops as paired evaginations from the endoderm and also the ectoderm of the third and fourth branchial clefts. It migrates caudally to reach the pericardium in human embryos of 12 to 19 millimeters. Its epithelial character is plainly evident until the end of the second month of uterine life in man, at which time the first thymic lymphocytes appear (*5*). Recent studies (*6*) indicate that the thymic lymphocytes arise by direct transformation of the epithelial cells, the mesenchyme providing only the initial inductive stimulus to differentiation and not contributing directly to the *initial* lymphoidal cell population.

If the lymphocytes in the thymus arise by direct transformation of the epithelial cells which originate at an ectodermal-endodermal junction, how do lymphocytes in other regions arise? Theoretically, they could originate independently of thymus lymphocytes, and in a similar way. This may be the case with the bursa of Fabricius (*7*) and possibly the appendix (*8*). However, according to morphologic studies, lymphocytes appear first in the thymus and only later in the circulation, spleen, lymph nodes, alimentary tract, and other tissues, and, in some species, appear there as late as the time of birth; they are numerous in the thymus and relatively scarce elsewhere until after birth; and they show practically no mitotic activity outside the thymus before birth (*4*, *9*). Thus it is reasonable to hypothesize that at least some lymphoid elements arise primarily in the thymus and subsequently migrate, at various times before or after birth, depending upon the species, to sites where they establish centers for the further development of lymphoid structures.

It is thus tempting to conclude that the development of immunologic responsiveness depends on the establishment of a functioning lymphoid system and that the thymus is the original source of at least some of the lymphoid elements concerned with immunologic reactivity. Experiments of various types are necessary to test the validity of this conclusion.

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Lymphocyte Production

Morphologic studies have suggested that, before birth, the thymus is the main, if not the sole, organ producing lymphocytes (*4*). What happens after birth? Throughout life in rodents the rate of multiplication of lymphoid elements in the thymus is about 7 times higher than that of lymphoid cells elsewhere (*10*). Even with advancing age lymphocyte production takes place in the involuted thymus with almost the same intensity as in the young thymus, and it is always greater in the thymus than it is in lymph nodes (*11*). The rate of production of lymphocytes in the thymus is unaffected by antigenic stimulation, resection of other lymphoid organs, partial resection of the thymus itself, or implantation of one or many thymus grafts in the same animal (*12*). Yet thymus lymphocytes are subject to the same general hormonal influences which affect lymphocytes throughout the body (*13*). These observations suggest that the strong stimulus for the proliferation of thymus lymphoid cells must arise from within the thymus itself and must be dependent on certain structures widely dispersed in the thymus. A spatial association between mitotic lymphocytes and specialized reticulum cells which stain with the periodic acid-Schiff reagent and which are distributed throughout the thymus cortex has indeed been described (*14*).

It is often assumed that lymphocyte production is one of the main activities of the lymph nodes. However, in the normal, unstimulated lymph node, the follicular centers are inactive, the cortical lymphocytic fields are devoid of mitotic activity, and immature lymphoid cells or blast cells are conspicuously absent (*15*). In germ-free animals the thymus is well developed and actively lymphopoietic but the lymph nodes are atrophic, with poorly formed follicles and no mitotic activity (*16*). It is now well established that one of the effects of antigenic stimulation is to increase the total cell population in the lymph nodes and spleen. A plasma-cellular reaction is induced; later, follicular centers, "secondary nodules," or "germinal centers" appear and become actively lymphopoietic (*15*). Mitotic activity of lymphocytes in lymph nodes thus apparently occurs only after antigenic stimulation.

The foregoing observations suggest that proliferation of lymphocytes is primarily a function of the thymus and that active lymphocyte production in

lymph nodes, and presumably in the spleen, occurs only as a result of antigenic stimulation. The cells proliferating in the thymus may be the precursors of some of the lymphocytes appearing in the circulation and in lymphoid tissues. On the other hand, the cells proliferating in the secondary nodules of the lymph nodes and spleen may be the precursors of cells which respond to a booster injection of antigen and which play some special role in the secondary response.

Lymphocyte Migration

The small lymphocyte is the commonest type of lymphoid cell circulating in blood and lymph. Experiments by Gowans (*17*) have clearly shown that the majority of thoracic duct lymphocytes are not newly formed cells but recirculating lymphocytes. When tritium-labeled small lymphocytes from the thoracic duct were transfused into the blood of a rat, a large proportion of them could be recovered from its thoracic duct lymph. Radioautographs showed that within 24 hours the cells had "homed" in large numbers into the lymphoid tissues—the cortex of the nodes, the white pulp of the spleen, the Peyer's patches, and the bone marrow. The only exception was the thymus, where few or no small lymphocytes appeared to lodge. The cells were shown to enter the cortex of the nodes by traversing the endothelial cells of the postcapillary venules and to leave again by way of the medullary sinuses and efferent lymphatics, thus entering eventually into the thoracic duct. Other experiments, in which tritiated thymidine was continuously infused into rats, showed that these small lymphocytes had a long life span (*18*), an observation obviously supporting the concept of recirculation. Accordingly, it appears that there is a pool of lymphocytes with a long life span that continually migrate from blood, through lymph nodes and other lymphoid structures, into lymph and back to blood, but that do not pass through the thymus.

Is there any evidence of a circulation of cells through the thymus? Studies on regenerating thymus grafts have shown conclusively that most, if not all, the lymphoid cells in the implant were ultimately derived from host cells (*19*), and not from thymus epithelial cells, as is believed to be the case in the embryonic thymus (*6*). Hence there may not be in the thymus, during postnatal life, an adequate or permanent population of special precursor or stem cells continually giving rise by mitosis to thymic lymphocytes. Instead, such a population may be continually replenished or replaced by immigrant cells entering from the circulation. By means of chromosome marker techniques it was found that marrow cells injected into a heavily irradiated host proliferated initially in both marrow and thymus, whereas injected thymus and lymph node cells proliferated only in lymph nodal tissue (*20*). The cells proliferating in the thymus had all the characteristics of thymus cells. The cells bearing the marker of the injected thymus and lymph node cells were replaced eventually by cells originally derived from the injected marrow cells. These experiments show that the irradiated thymus will receive cells from the circulation, and suggest that such cells then differentiate exclusively along lymphoid pathways. Stem cells or lymphoid precursor cells in the marrow inoculum may thus have either to pass through the environment of the thymus or to receive some factor elaborated by the thymus in order to differentiate to lymphoid cells. These possibilities are made all the more likely by the results of experiments obtained with thymectomized irradiated mice injected with marrow cells. The lymphoid tissues of such mice failed to regenerate (*21*).

Strong evidence for an afferent stream of cells to the thymus thus exists. Do lymphocytes ever migrate out of the thymus? Clearly some sort of lymphoid cell must come out of the thymus if the hypothesis that the thymus is a primary source of lymphocytes is to be held. Experiments in which thymus cells were labeled *in situ* with tritiated thymidine showed that surprisingly small numbers of labeled cells seeded out of the organ (*22*). These experiments were performed in guinea pigs, a species in which significant lymphocyte migration from the thymus, if it occurs, may take place well before birth.

The simplest hypothesis would then be that the thymus is an important source of circulating small lymphocytes. The lymph nodes, on the other hand, would provide both a meeting point for circulating lymphocytes and antigen brought there by afferent lymph and a site for the subsequent proliferation of those cells that have interacted with antigen. Under normal conditions after birth, the thymus may have little to contribute to the circulating pool, owing to the long life span of the circulating cells. In the antigenically stimulated animal, the secondary lymphoid nodules in the spleen and nodes may contribute to the circulating-pool cells capable of mediating secondary immune responses. The constant stimulus to cell proliferation in the thymus may act as a built-in mechanism to safeguard the animal against depletion of lymphocytes such as occurs under conditions of stress. During recovery from stress, the thymus might be required again to build up the pool of circulating lymphocytes, presumably by direct cellular contribution.

Immunologically Reactive Cells

It has been known for a long time that the spleen and lymph nodes play an active part in immune reactions. Thus, after antigenic stimulation, both a high antibody titer and numerous antibody-secreting plasma cells can be demonstrated in these organs (*23*). Recently, suggestive evidence has been obtained indicating that some primary immune responses may be initiated by the interaction of antigen—or antigen which has been processed by reticuloendothelial macrophages (*24*)—with circulating small lymphocytes (*25*) or, in some cases, with large lymphocytes (*26*). After interacting, these cells would settle in the cortex of the nodes or white pulp of the spleen and establish a dividing cell line which generates the effector cells responsible for production of antibodies or destruction of a foreign graft.

The thymus, which is outside the path of recirculation of immunologically competent lymphocytes, does not show production of antibody, plasma cells, or germinal centers in an immunized animal (*27*), thus behaving quite differently from other lymphoid structures. This implies either that there are no cells in the thymus capable of mediating immune reactions or that, if such cells exist, antigen is not made available to them. Direct injection of antigen into the thymus parenchyma was associated with the appearance of antibody-producing plasma cells and the formation of germinal centers within the thymus (*28*). This finding suggested that the failure of the intact thymus to react to circulating antigen might be due in part to the existence

of a barrier between blood and thymus lymphocytes which prevents entry of antigen into the thymus. The possibility that cells of the circulating pool may have accumulated within the injection site and initiated the reactions there was not, however, excluded. In sections of the thymus viewed by electron microscopy it was found that the lymphocytes lay in a space separated from connective tissue and blood vessels by a more-or-less continuous layer of epithelial cells and basement membrane (*29*). This layer could, conceivably, function as a hematothymic barrier. However, other experiments, in which thymus cells were transferred to appropriate recipients, suggested that there may be few, or only weakly reactive, cells in the thymus. Thus, for instance, rat thymus cells could not produce a graft-versus-host immune attack when injected into a foreign newborn host. Mouse thymus cells could produce such a reaction, though less effectively than mouse lymph node cells (*30*). Furthermore, thymus cells taken from presensitized donors, or from normal donors, and then incubated in vitro with antigens have produced little or no antibody in appropriate recipients, in complete contrast to nonthymic lymphoid cells (*31*). The failure of the thymus to react actively to antigenic stimuli may thus be due not only to a postulated hematothymic barrier restricting the entry of antigen into the thymus but also to the paucity or absence of cells capable, in their present state, of reacting immunologically. If the thymus provides at least some of the cells of the circulating pool, then some change must occur in its cells, after they leave the organ, that makes them immunologically competent.

Effects of Thymectomy

If the thymus directs the development of immunologic responsiveness it might be expected that its removal would lead to defective immunologic performance. Thymectomy of rodents in adult life was accompanied by a drop in lymphocytes (*32*) but not by any significant impairment of immune functions (*33*). Thymectomy at or soon after birth, on the other hand, was associated with much more serious defects (*34–36*). The lymphocyte population in blood and tissues (except marrow) became severely diminished by 1 to 2 months after birth, and the capacity to produce an antibody response to some, but not all, antigens, to develop delayed hypersensitivity, and to reject foreign skin grafts was markedly impaired. In mice there was evidence of extramedullary hematopoiesis and hyperplasia of reticuloendothelial elements (including Kupffer cells), and the phagocytic activity, as measured by the clearance of blood carbon, was increased (*37*). The serum levels of 7*S* and 19*S* gamma globulins were within normal limits, but the levels of beta globulin 2*a* (gamma globulin 1*a*) were elevated in thymectomized mice (*38*). In rats there was an absence, or a deficiency, of an immunoglobulin designated IgX, which extends cathodally into the slow β region and anodally well into the α region (*39*). Thus the defects in formation of antibody to some antigens in thymectomized rodents cannot be attributed to a failure either of phagocytosis or of synthesis of 7*S* or 19*S* gamma globulin. There may, however, be a specific defect in the formation of one of the species of immunoglobulins, at least in the rat (*39*).

Mice of many strains thymectomized at birth, or not later than 48 to 72 hours after birth, developed a fatal wasting syndrome characterized by loss of weight, ruffled fur, hunched posture, and diarrhea; this occurred between 1 and 4 months of age, depending on the strain (*34, 40*). The pathogenesis of this syndrome is still unclear. A similar syndrome has been described in hamsters thymectomized at birth (*36*). It must be emphasized that the defects in immunologic responsiveness that were found in many strains of mice after thymectomy were evident well before the clinical onset of the wasting syndrome (*34*).

Thymectomy of the mouse at 1 to 2 weeks of age was still associated with some impairment of homograft immunity, but only when donors and hosts were closely related genetically. Mice subjected to a sham operation at birth or to partial thymectomy behaved like completely normal mice (*34*).

Hence it is evident that, when thymectomy is performed before the full development of the immunologic apparatus, severe defects in lymphopoietic and specific immune functions become evident. This is in marked contrast to the negligible effects of thymectomy when it is performed later. It may thus be concluded that the role of the thymus is that of establishing the cellular basis for a normally functioning immune system, and that this function is restricted to a short period following the development of the organ itself. Thymectomy in adult life causes no serious defects, partly because the cells in the circulating pool have a long life span and partly because the nodules in the peripheral lymphoid organs are sufficiently well established and self-supporting to cope with any antigenic stimulus. What would happen, however, if massive destruction of lymphocytes were to take place in an adult animal? Adult mice thymectomized and subsequently exposed to total-body irradiation showed severe defects in their lymphocyte population and immunologic functions. On the other hand,

Table 1. Protection against the effects of thymectomy in newborn mice.

Treatment 1 week after thymectomy	Number of mice	Number of mice in three groups according to age at death			Percentage of protection (% of mice surviving beyond 4 months)	Number of mice grafted with skin*	Number of mice rejecting graft
		30–60 days	60–120 days	> 120 days			
None †	16	14	2	0	0	4	0
Lymph node cells †	21	2	2	17	81	19	18
Spleen cells †	13	1	2	10	77	13	11
Bone marrow cells ‡	15	4	10	1	7	12	1
Thymus cells from newborn mice ‡	16	5	11	0	0	10	0
Thymus cells from adult mice ‡	14	2	6	6	43	10	6
Saline thymus extracts ‡	15	2	13	0	0	13	0
Graft of thymus from newborn mouse ‡	18	1	3	14	72	15	15
Graft of thymus from 2-week-old mouse ‡	10	1	2	7	70	8	6
Thymus in chamber ‡	14	1	1	12	85	12	8

* Donor of skin graft, strain Ak. † Donors and hosts, strain C57BL. ‡ Donors and hosts, strain CBA.T6.

Table 2. Restoration of immune function in adult mice (strain CBA) thymectomized and irradiated.

Treatment	Number of mice	Number of mice showing skin graft * survival for			
		< 20 days	20–40 days	40–70 days	> 70 days
None	20	20	0	0	0
Thymectomized, irradiated, and injected with 40 million bone marrow cells †	11	0	0	6	5
Thymectomized, irradiated, and injected with 10 million spleen cells †	40	30	10	0	0

* Donor of skin graft, strain C57BL. † From strain CBA mice.

controls subjected to sham thymectomy and then exposed to total-body irradiation recovered, within 4 weeks, normal lymphocyte levels and the capacity to produce all types of immune responses (*21*). These experiments clearly show that, in the adult, the thymus is still required for reestablishing immune mechanisms when the immune apparatus has been damaged or destroyed.

Protection against Effects of Thymectomy

A number of proposed methods of protecting mice against the effects of thymectomy at birth were tested. An injection of 5 million spleen or lymph-node cells, but not of thymus cells from newborn mice, was effective in giving protection when administered to mice of the same strain a few days after thymectomy (*34, 41*) (Table 1). In mice so treated, immune reactions occurred just as in normal mice, and the treated mice did not develop wasting disease. They still, however, showed some diminution in their lymphocyte population. In contrast to spleen cells, marrow cells completely failed to restore immune functions in mice thymectomized at birth or in adult mice thymectomized and irradiated (*21*) (Table 2). In the marrow suspensions used, 20 percent of the cells were classifiable morphologically as small lymphocytes. Mice injected with 10 million spleen cells recovered the capacity to produce immune responses, but those injected with as many as 40 million bone-marrow cells (8 million of which appeared morphologically as small lymphocytes) failed to show such recovery (*21*). Functionally, apparently, the small mononucleated marrow cells, lymphocytic in appearance, must be different from the small lymphocytes of the circulating pool.

Mice thymectomized at birth and grafted within a week with an intact thymus, or part of one, from a donor aged from 1 day to 2 to 3 weeks, developed normally, did not suffer from wasting disease, had normal populations of lymphocytes, and could reject foreign skin grafts as normal mice do (*34, 41*) (Table 1). It was tempting to conclude that the thymus implant was producing the lymphocytes which were missing in the thymectomized host and which would colonize the host's lymphoid tissue, thus producing a lymphoid chimera. To test this possibility, donor-and-host combinations were used in which host cells could be identified at metaphase by the presence of an unusually small chromosome (*41*). This led to the finding that the great majority of cells multiplying in the lymphoid tissues and in the thymus implant itself were of host origin and could thus not have been the direct descendants of cells of the thymus-donor strain. Further tests in which discriminant spleen assays were used showed that host cells were largely responsible for immunologic reactivity in the mice in which the immune response had been restored (*42*). It was thus obvious that the role of the thymus implant was not just to provide directly the cells which populated the lymphoid tissues of the thymectomized host but to exert some influence on the host's own lymphoid system. This influence was particularly striking in adult mice that had been thymectomized, heavily irradiated, injected with labeled marrow cells, and grafted under the kidney capsule with allogeneic thymus tissue (*43*). The epithelial-reticular framework of the thymus graft was never repopulated by lymphoid cells, and the graft survived for only 10 to 15 days. The lymphoid tissues of the host, on the other hand, had dividing cells of marrow-donor type but never of thymus-donor type. The mice eventually recovered the ability to produce immune reactions and could reject skin of thymus-donor type as a second-set response (Table 3) (*43*). Clearly, therefore, within a very short period an allogeneic thymus implant induced differentiation of immunocompetent cells in a population of cells arising from the marrow inoculum and sensitized the regenerating lymphoid system against the histocompatibility antigens of the foreign implant itself.

In order to see whether a direct cellular transfer between thymus implant and thymectomized host was at all necessary for protection, thymus tissue was placed in Millipore diffusion chambers inserted into the peritoneal cavity of young mice thymectomized at birth. These mice gained weight satisfactorily and did not develop wasting disease (*44, 45*) (Table 1). They could also produce serum antibodies and re-

Table 3. Effect of thymus implants on skin homograft survival in thymectomized irradiated mice, strain CBA.

Treatment	Strain of skin donor	Number of mice	Number of mice showing skin graft survival for			
			< 20 days	20–40 days	40–70 days	> 70 days
Thymectomized, irradiated and injected with 5 million marrow cells from strain CBA mice	Ak	37	0	0	0	37
	C57BL	38	0	0	0	38
Thymectomized, irradiated, injected with 5 million marrow cells (strain CBA) and implanted with thymus (strain CBA) under renal capsule	Ak	19	3	10	6	0
	C57BL	21	12	9	0	0
Thymectomized, irradiated, injected with 5 million marrow cells (strain CBA) and implanted with thymus (strain C57BL) under renal capsule	Ak	22	1	2	4	15
	BALB/c	11	6	3	2	0
	C57BL	22	20*	2	0	0

* Rejected as a second-set response.

ject allogeneic skin grafts (*44*). The extent to which their lymphoid tissues were populated was, however, variable: some mice had perfectly normal lymphoid structures whereas others still showed a diminution of lymphocytes in spite of having produced normal immune responses to sheep erythrocytes and skin homografts (*44*).

These experiments suggest that some of the action of the thymus may be mediated by means of a humoral factor. An ability of thymus extracts to produce a lymphocytosis has been claimed by many workers (*46*), but to date there is no evidence that such extracts protect neonatally thymectomized mice (*41*) (Table 1).

Immune Tolerance and Autoimmunity

A specific inhibition of the immune response can be induced in normal animals by injecting a particular antigen at birth. It can also be produced in adults by injecting large doses of antigen, or by injecting the antigen after treatment of the recipient with irradiation or radiomimetic compounds. This state of inhibition of the immune response ("immune tolerance") will persist as long as the concentration of the antigen remains above a certain critical level. When the concentration drops below this level, tolerance "breaks," and the capacity to respond to the antigen reappears (*47*). Does the thymus play any role in acquired immune tolerance? Recent experimental evidence suggests that it is concerned with the breakdown of tolerance. It has been shown that whole-body irradiation accelerates the breakdown of a tolerant state (*48*). This presumably occurs as a result of the differentiation of new immunocompetent cells during the recovery phase. Since the thymus is essential for the recovery of immune functions after total-body irradiation (*21, 43*), it seems likely that it is also responsible for the breakdown of tolerance under these circumstances. This has in fact been demonstrated, and it has been shown further that thymectomy in an adult tolerant to a specific antigen prevented the reappearance of reactivity with respect to that antigen (*49*). The breakdown of tolerance must thus occur by means of a thymus-dependent mechanism which allows the development of new uninhibited cells.

Permanent autotolerance may, like artificially induced tolerance, depend on the fact that lymphoid cells are constantly in the presence of autoantigens. Since the thymus plays a role in the breakdown of induced tolerance, is it not possible that it is also responsible for the breakdown of autotolerance, and hence for the development of autoimmune diseases? Evidence suggesting that the thymus induces the breakdown of autotolerance has been obtained in mice. Normal mice thymectomized at birth and grafted with thymus from a strain of mice with a genetically determined autoimmune disease themselves developed autoimmune phenomena (*50*).

The thymus of mice with autoimmune hemolytic anemia showed numerous germinal centers, lymph follicles, and accumulation of plasma cells and mast cells (*51*). Similar lesions as well as thymus tumors have been described in patients with myasthenia gravis, systemic lupus erythematosus, rheumatoid arthritis, aregenerative anemia, and other diseases thought to have autoimmune manifestations (*52*). These findings led Burnet (*53*) to postulate that the thymus may be the seat of origin of clones with autoimmune potentialities and that, under normal circumstances, these clones are eliminated within the thymus by some homeostatic mechanism. No experimental evidence has, however, been produced to show that the abnormal collections of cells in the thymus represent the actual source of "forbidden clones." The lesions in the thymus may occur simply because the thymus is one of the target organs. In "autoimmune" disease states, germinal centers have also been found in other sites where they usually do not occur—for example, in the salivary glands in Sjögren's disease, in the skin in cutaneous lupus, in the thyroid in Hashimoto's disease, in the colon in ulcerative colitis, in the knee joint in rheumatoid arthritis, and in the liver in lupoid hepatitis (*54*).

If an autoimmune disorder is due to the development of "forbidden clones" in some part of the thymus, then thymectomy should alleviate or cure the autoimmune condition. Thymectomy in patients with myasthenia gravis may alleviate the symptoms in certain selected cases, and it often, but not always, tends to diminish the magnitude of the serologic abnormalities (*55*). On the other hand, two cases have been reported in which systemic lupus erythematosus and other disorders, possibly autoimmune in nature, have developed after thymectomy for myasthenia gravis (*56*). Thymectomy in newborn mice with genetically determined autoimmune disease did not prevent but, rather, accelerated development of the disease, inducing it in a more acute and florid form (*50*). The fact that thymectomy, in some well-documented cases, has aggravated autoimmune disease suggests that the thymus may exert some control over abnormal immunologic reactivity. The disease may progress because, as a consequence of damage to the thymus, a reduction of the normal control over immunologic reactivity occurs. More experimental work is clearly required to elucidate the role of the thymus in autoimmunity.

Neoplasia

Evidence has been accumulating which shows that many chemically induced and virus induced tumors are antigenic in their hosts and that resistance against such tumors, which is mediated by lymphocytes, can be demonstrated (*57*). Since the thymus exerts some influence over the development of cells which initiate various types of immune reactions, it must play some part in providing resistance to tumor growth. In fact, mice, rats, and hamsters thymectomized at birth are more susceptible to carcinogenic agents than normal animals are (*58*). For instance, mice of strain C57BL are normally resistant to the oncogenic activity of polyoma virus, even if they are inoculated with the virus at birth. On the other hand, C57BL mice thymectomized in the neonatal period are markedly susceptible to the virus (Table 4). These results suggest that antigenic tumor cells are more likely to grow successfully when the cellular immune mechanism is impaired. Only weakly antigenic or nonantigenic tumors, or antigenic tumors from which there is inadequate release of antigen, should ever become successfully established.

Discussion

The neonatally thymectomized animal presents an enigma: it develops more or less normally for about 1 month but eventually shows a marked diminution, yet never a complete absence of small lymphocytes in blood and tissues; it lacks the capacity to

produce some immune reactions yet is not deficient in most of the species of immunoglobulins and it usually dies from a syndrome the pathogenesis of which has not yet been fully elucidated.

Although thymectomized mice eventually show a marked diminution of small lymphocytes their lymphoid tissues do develop to a certain extent during the first few weeks after birth (*34, 37*). The newborn mouse already has circulating lymphocytes and diffuse accumulation of lymphocytes in the spleen. The organization of such cells into lymphoid structures may be dependent not on the thymus but on extrinsic (antigenic?) stimuli encountered after birth. On the other hand, there may be organs other than the thymus which control some of this early lymphoid development, organs like the bursa of Fabricius in the chick (*7*) or the appendix in the rabbit (*8*).

The antibody response to some standard antigens is reduced after thymectomy in the newborn animal (*34*). However, very few thymectomized mice were unable to respond to at least one antigen in a mixture of three administered simultaneously (*38*). In relation to a clonal-selection theory of immunogenesis, this finding implies either that the precursors of those cells responsible for the antibody response had differentiated prior to the time of thymectomy or that their differentiation depended not on the thymus but on other organs.

In thymectomized mice which were unable to reject skin grafts, small lymphocytes occurred in reduced numbers but were never completely absent (*34, 41*). Mice with as many as 5000 lymphocytes per cubic millimeter have failed to reject skin grafts. It is possible that these mice still had some cells competent to deal with the graft, but in numbers too small to produce an adequate response. On the other hand, it is possible that the majority of these cells belonged to an entirely different population of cells—a population similar in this respect to that in the bone marrow, which failed to restore immune functions in thymectomized mice.

The fact that the extent of depletion of lymphocytes increases with age in the neonatally thymectomized mouse suggests that the thymus may be essential to maintenance of the normal complement of lymphocytes in the body. Three factors any or all of which may be responsible for the eventual atrophy of a lymphoid system that can receive no further contribution from the thymus are, (i) exhaustion as a result of constant antigenic stimulation; (ii) disintegration due to the activity of autoimmune clones arising in the absence of some thymic control; and (iii) destruction as a result of some process associated with infection. No direct evidence is available to determine which of these factors is playing a role.

Table 4. Effect of thymectomy on the induction of tumors by polyoma virus.

Treatment		Number of mice	Hemagglutination inhibiting antibody titers at 5 to 8 weeks of age	Mice with parotid tumors	
Operation at 3 days	Polyoma virus at 4 days (plaque-forming units)			Number	Latency period (days)
Sham thymectomy	2×10^6	16	100–3200 (av., 1600)	1	118
Thymectomy	2×10^6	20	100–3200 (av., 800)	18	52–78 (av., 69)
None	0	8	<100	0	

The striking resemblances between the neonatal-thymectomy syndrome and graft-versus-host diseases have been stressed (*34, 37*). The major points of similarity between the two conditions are as follows: hyperplasia of the reticuloendothelial system; proliferation of reticulum cells, histiocytes, and Kupffer cells; increased phagocytic activity; extensive extramedullary hematopoiesis; scattered necrotic leucocytes; diminished immunologic competence; lymphoid aplasia, with wasting and death. Such changes in the neonatally thymectomized mouse, by analogy with changes in the mouse in which a graft-versus-host reaction has been induced, may be the result of some immune, and thus necessarily autoimmune, reaction. The immunologic incompetence associated with thymectomy in the newborn may be the result of an "immunologic preoccupation" of the lymphoid cells with an autoimmune process. There is, however, no direct evidence for the operation of autoimmune processes in thymectomized mice. On the other hand, the wasting syndrome associated with thymectomy in the newborn has never been observed in inbred mice kept in the germ-free state (*59*). This indicates that an infectious process must play some role in the pathogenesis of the wasting syndrome.

Evidence has been presented to show that the immunologic responsiveness of thymectomized mice can be restored to normal by means of a humoral thymus mechanism. A humoral thymus factor could conceivably act on lymphocytes or on lymphoid precursor cells in one of two ways. If a few immunocompetent cells were left after thymectomy, the factor might induce them to multiply to such levels that an adequate response would become possible. The factor would stimulate lymphocytosis in a manner similar to the action of the lymphocytosis-stimulating factor of Metcalf (*46*). The high proliferative rate of lymphocytes in the thymus may be the direct result of the stimulating action of such a factor. It seems unlikely, however, that it would induce lymphopoiesis in lymph nodes and spleen, for, as mentioned above, in the absence of antigenic stimulation, lymphopoiesis outside the thymus is minimal or absent. On the other hand, the thymus factor might be responsible for the differentiation of lymphoid precursor or stem cells into cells capable of reacting immunologically. At a certain stage of ontogenesis, lymphoid cells, or their precursors, would have the genetic information necessary for synthesizing globulin molecules but not the capacity to express this information and thus to respond immunologically. It may be that this capacity is acquired only after the cells have been acted upon by the postulated thymus factor. Acquisition of this capacity would thus represent a further step in the differentiation of cells of the lymphoid series. In the normal animal, this step may be initiated within the thymus, and this may be followed by the emigration of the differentiating cells. Under certain experimental circumstances, described above, differentiation of the lymphoid cells would apparently occur outside the thymus environment.

Summary and Conclusions

Recent experiments have made it clear that the thymus plays a key role in directing the establishment of a functioning immunologic apparatus. Thymic ablation in early life, before the immune system is fully developed, is associated with disturbances in im-

mune functions. In marked contrast are the negligible effects of thymectomy in adult animals in which immunologic mechanisms are presumably well established. If, however, the cellular basis for such mechanisms is destroyed—for example, by ionizing radiations—thymectomy is again associated with serious defects in immune functions. Thus, the thymus is necessary not only for establishing normal immunologic potential during development but also for restoring such potential after it has been destroyed or damaged, and possibly for maintaining it as it becomes depleted with time. Further work is needed which will enhance our understanding of the relationship of the thymus to the remainder of the lymphoid complex—of its precise role in directing the activities of lymphoid cell populations which underlie such phenomena as immunologic responsiveness, inhibition of the immune response, autoimmunity, and, possibly, natural resistance against cancer. Evidence shows that a humoral thymus mechanism is involved in the maturation of immunologic capabilities. A humoral factor may be required either for stimulating the proliferation of immunocompetent cells or, as seems more likely, for inducing the differentiation of lymphoid precursor or stem cells to lymphoid cells, which can then participate in immune reactions. Further understanding of the nature of the defects occurring after thymectomy and of the mode of action of a humoral thymus factor must await the development of in vitro systems in which the response to antigenic stimuli can be studied at the cellular level.

Immunologic abnormalities in man are often associated with thymus lesions. Thus, thymus atrophy and thymus tumors have been reported in cases of hypogammaglobulinemia and in various types of immunologic deficiencies (*60*). Hyperplastic lesions and tumors of the thymus often occur in disorders having autoimmune manifestations (*52*). The possibility that disturbances in thymus function may be intimately involved in the pathogenesis of such diseases must be seriously explored. Extraction and purification of a thymus factor responsible for controlling the development of immunologic reactivity may lead to its use in clinical medicine and, hopefully, to the conquest of some of the diseases of the immunologic system.

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Genesis of the Arctic Ocean Basin

High-altitude aeromagnetic surveys provide new data on the earth's crust in this remote area.

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Aeromagnetic surveys have been systematically made of more than half the Arctic Ocean, and the high-altitude aeromagnetic profiles thus obtained (*1*) reveal significant contrasts in the magnetic character of its several parts. These contrasts suggest that the earth's crust under the Arctic Ocean is complex, and that it resulted from profound geologic changes dating back to Precambrian time.

The Arctic Ocean has the distinction of being the last of the oceans to be discovered. Pack ice blankets its surface, and the entire region was thought to be covered by shallow seas until Nansen's polar expedition of 1899 recorded water depths of oceanic magnitudes. As more soundings were made, it was found that the deep water is confined to a relatively restricted central area that is surrounded by continental shelves which are exceptionally broad off the Eurasian coast (Fig. 1). This deep water is completely blocked off from the deep water of the Pacific and is linked with the Atlantic by a single, narrow trough between Greenland and Spitsbergen. Not until the end of World War II was it discovered that a major submarine mountain range, the Lomonosov Range, extends across the entire basin, dividing it into two parts. The basin on the North American side is further broken up into two flat-floored basins by a lower but much broader submarine feature, the Alpha Rise. Although a considerable part of the basin on the Eurasian side of the Lomonosov Range is also flat-bottomed, there is a large area adjacent to Spitsbergen and Franz Josef Land which has very rugged bottom relief. Much uncertainty still remains concerning the detailed topography under the Arctic Ocean, although the advent in 1957 of nuclear submarines able to obtain continuous bottom profiles has contributed greatly to knowledge of the terrain on the sea floor.

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Nature of the Arctic Ocean Basin

The nature of the Arctic Ocean Basin is a subject of debate, primarily between geologists working on the adjacent continental areas and geophysicists utilizing data from earthquake seismology and various types of geophysical measurements over the basin area itself. For the earth as a whole, seismic refraction measurements, supported by much more abundant gravity data, show that the crust in the continental areas is radically different from that in the oceanic areas. The Mohorovičić discontinuity, which separates the dense, high-velocity rocks of the mantle from the overlying crust, is 20 to 40 kilometers deep under the continents and only about 5 kilometers deep under the floor of the oceans. The continental crust is predominantly silicic material in which there is an increase in density and in seismic velocity with depth, whereas the oceanic crust is composed of more mafic rock under a thin veneer of sedimentary material. Ewing and Press (*2*) state that, with the exception of certain marginal tectonic belts, the earth's crust appears to belong to one of these two distinct types. So far, direct measurements of crustal thickness have not been made in the Arctic Ocean Basin. Until the oceanic or continental nature of this region has been definitely established, valid conclusions about its geologic history and the interrelationships of the surrounding continental blocks cannot be made.

Geology of Surrounding Areas

The known geology of the surrounding areas has led many geologists to conclude that the deep Arctic basins are a later development in a region which was formerly an integral part of the North American and Eurasian continents. A large part of this region comprised the Hyberborean Shield of Shatskiy (*3*) and constituted the nucleus of Eardley's proposed "Ancient Arctica" (*4*), for which he cites a number of arguments that are briefly summarized here.

The Precambrian shields of the Northern Hemisphere, which form the cores of the continents, cluster around the Arctic like fragments of