The Endocrine Thymus

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ABSTRACT

The mediastinal, lympho-epithelial organ was termed thymus to denote a ductless, gland-like body which has the appearance of a "warty excrescence" (Greek, thymos). Early investigators had ascribed a functional role to this gland including the production of humoral factors which modulated cell growth, maturation and exerted influence on the process of mineralization. Investigators of the past decade have vindicated these observations and identified that the thymus is, indeed, an organ which "sets in motion, and spurs on" (Gr. hormaein) target tissues.

Introduction

In 1942, Maximow and Bloom, in their textbook of histology, summarized the knowledge available on the function of the thymus gland. They wrote: "The functions of the thymus are unknown, except for its ability to form lymphocytes, a few plasma cells and myelocytes. That the change from a very large organ in the embryo, infancy and childhood, into a gradually disappearing organ with development of sexual maturity, has led many authors to ascribe an endocrine function to this gland. The claims that the injection of extracts of the thymus into parent rats causes a precocious growth of their progeny which is cumulative in succeeding generations, and that thymectomy causes a retardation in growth of the progeny have not been substantiated."

Until the early 1960's, the available information dealt with embryological development, a description of histologic components and a recognition of aberrations of morphology. This organ is derived from the third and fourth branchial clefts which yield its epithelial components, while the lymphoid structures develop later in fetal life. Its cortex contains closely packed thymic lymphocytes surrounding a medulla composed of lymphocytes and epithelial reticular cells including Hassall's corpuscles.

Changes in structure were reported in certain endocrine disturbances and in malignant transformation; there was no direct evidence that the organ produced any internal secretion. Atrophy of the gland was a physiologic process occurring after puberty or could occur precociously following serious illnesses or infections.
in childhood. Hyperplasia of the gland occurred when associated with endocrine disorders such as thyrotoxicosis, acromegaly and, in some instances, Addison’s disease. Historically, marked enlargement of the thymus, status thymicolympathicus, was said to produce respiratory compression symptoms of dyspnea, cyanosis, etc., in the vulnerable infant. This concept of a constitutional abnormality characterized by enlargement of the thymus was subsequently recognized to play no role in the disease manifestations; however, the functional significance of immunoincompetence in such children warrants reinvestigation. Neoplasms of this gland are relatively rare; however, malignancies of the epithelial and lymphoid components have been documented.

The rapid advances in the study of immunobiology in man, the delineation of the T and B cell systems, and the association of thymic tumors with abnormalities of neuromuscular transmission have resulted in a reexamination of the hormonal function of the thymus.

Thymic Hormone Physiology and Chemistry

Earlier investigators had reported that thymectomy affected mineralization and the growth of experimental animals. Evidence had been accumulated identifying a relationship between defective mineralizations of eggs in doves which was corrected by treating these birds with a thymic extract.

Definitive experimental data establishing the endocrine function of the thymus was the result of studies in experimental animals and as a consequence of investigation of the pathophysiology of human diseases. Hallmark experiments on neonatal thymectomy in mice and other animal species produced profound impairment of the immune system, lymphocyte deficiency in the peripheral blood and reticuloendothelial system, as well as the development of a “running” syndrome. Shortly after the description of the effects of thymic ablation, laboratory investigations demonstrated reversal of this symptom complex by subcutaneous implantation of thymic tissue (or intraperitoneally) and finally by the administration of thymic cell free extracts.

These experimental data established an endocrine role of the thymus gland and related its action to the ontogenesis, function and senescence of the T-lymphatic system. It was predictable, based on these observations and the functional role of the T cell system, that defects would be encountered in man relating thymic abnormalities with immunodeficiency, autoimmune or neoplastic diseases. Subsequent investigations by Goldstein and collaborators, Metcalf and others have defined abnormalities in thymic endocrine secretory function in a variety of disorders.

Essential to a discussion of thymic hormone physiology and chemistry is a review of certain aspects of T-cell immunobiology. T-cells are the thymic modified or derived mononuclear cells which are functional in the defense against fungal and viral infections, in the host-versus-graft response and in the development of delayed hypersensitivity or cellular immunity. The progenitor T-lymphocytes originate from the bone marrow and migrate to the thymus gland where they subsequently undergo immunologic transformations. Following their passage through the thymus gland, they undergo a process of differentiation resulting in the development of immunocompetence. Subsequently, they migrate to peripheral lymphoid organs where they coexist with B-lymphocytes. Animal experiments employing thymectomy and thymus implantation, or administration of thymic cell-free extracts, identified that the gland itself or its products are necessary for the maturation of T-lymphocytes. The thymus may be viewed as regulating the
maturation of the marrow precursor cell to a thymocyte and, subsequently the differentiation of the thymocyte into a mature immunocompetent cell. The in vitro and in vivo monitoring of these maturational changes is dependent upon the application of a methodology which identifies a T-cell and assesses its immunocompetence.

The major assays for the recognition of immunocompetent cells are: blastogenic transformation, mixed lymphocyte reaction, the in vivo mixed lymphocyte test or graft-versus-host reaction, measurement of thymic membrane antigens and the demonstration of immunoglobulins on the surface of thymocytes.

Blastogenic transformation of T-cells is assessed through the use of two mitogens, phytohemagglutinin and concanavalin A. Following addition of either mitogen to a suspension of mononuclear cells the incorporation of $^{3}H$-thymidine into macromolecular deoxyribonucleic acid (DNA) is monitored. This assay gives an index of the maturation of T-cells since immunoincompotent cells do not show increased DNA synthesis. The second methodology, the mixed lymphocyte reaction, evaluates the competence of immunocompetent T-cells to undergo blastogenic transformation following exposure to lymphocytes (mononuclear cells) from another subject. Here too, DNA synthesis is monitored by the incorporation of $^{3}H$-thymidine. In vivo assessment of immunocompetent T-cells is measured in the graft-versus-host reaction (in vivo mixed lymphocyte test). Donor T-cells are injected into an immunosuppressed experimental animal (immunologic immaturity in the newborn animal or artificial suppression of the host immune system). Induction of splenomegaly occurs in the host as a consequence of the presence of immunocompetent donor T-cells.

A technological advance in the study of immunocompetence occurred with the recognition of a quantitative increase in cell surface antigens of T-cells as they mature. Those cell surfaces antigens used as an index of immunologic competence are: THY-1 antigens, TL-antigens, LY-antigens (in the mouse the H2 locus) and the HLA locus in man. Investigations of the structural characteristics of these cell surface antigens has been carried out by many investigators.

The cardinal experiment which established the existence of thymic humoral factors was the restoration of immunological competence in neonatally thymectomized mice by cell free extracts. Evidence for the in vivo production and function of these humoral factors was the observation that neonatally thymectomized female mice had restoration of normal immunological manifestations during pregnancy which could only be explained by the transfer of "thymic" factors produced by the fetus. The described effects of these hormones include: (1) an increase in the level of circulating lymphocytes as well as increased lymphocytes in certain areas of the reticuloendothelial system, (2) restoration or repair of cellular immunity, (3) regulation of antibody production to certain thymus-dependent antigens, and (4) maintenance of resistance to infection, particularly those of viral origin. The macromolecular thymic factors whose identity or functional significance is now fairly well established are shown in table I.

The best studied thymic hormone is thymosin, having a molecular weight of approximately 10,000 and composed of 108 amino acids, half of which are dicarboxylic amino acids. This polypeptide was administered to thymectomized animals, produces restoration of the graft vs. host reaction and accelerates the immunological maturation of T-cells. Presumptive data imply that thymosin exerts its activity through a cAMP-mediated mechanism. The specificity of

* Adenosine 3', 5'-cyclic monophosphate.
Biological Activity of Other Thymic Factors

| TABLE I

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<th>Biological Activity of Other Thymic Factors</th>
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<td>Lymphocytosis Stimulating Hormone (LSH&lt;sub&gt;R&lt;/sub&gt;, LSH&lt;sub&gt;H&lt;/sub&gt;)</td>
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<tr>
<td>Homeostatic Thymic Hormone</td>
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<td>Thymic Humoral Factor</td>
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<td>Thymosin</td>
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<td>Thymopoietin</td>
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<td>Thymic Lipid Fraction (Fr. B, Fr. B III)</td>
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<td>Thymosterin</td>
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Thymosin action is questioned since a number of chemicals which increase intracellular cAMP levels may induce differentiation of precursor T-cells in vitro.

Thymopoietin is yet another peptide hormone(s) isolated from this gland. Two molecular species of this peptide hormone have been isolated from mouse thymus. These macromolecules, of molecular weight 5500 and 1600, respectively, increase the responsiveness of precursor T-cells to lymphoblastic transformation with concanavalin A and phytohemagglutinin. In vitro incubation of precursor cells with these peptide hormones results in the acquisition not only of T-mitogen responsiveness but also the acquisition of the THY-1 antigen. Though these thymopoietins have similar biological activities, thymopoietin 2 appears to be only 3 percent as biologically active as thymopoietin 1. Most important is the demonstration that thymopoietin 1 and 2, when injected into mice, induced impairment of neuromuscular transmission similar to that observed in myasthenia gravis.

Thymic humoral factor has been isolated from human and murine serum. Its biological activity in crude serum and purified fractions is assayed by the induction of rosette formation following incubation of sheep erythrocytes in the presence of spleen cells or T-cells bearing the THY-1 antigen. Specificity is given to this bioassay since rosette formation is inhibited when THY-1 antigen-bearing cells are incubated in the presence of azathioprine. The in vitro thymic rosette-inducing effect is exerted within a short period of time and at nanogram concentrations of the purified peptide.

Experimental details on the structure, preparation and biological activity of the other thymic factors (table I) may be found in the recent literature. Recent investigations support the concept that the thymus produces several factors involved in the maturation of precursor cells into immunocompetent T-cells. Advances in the study of the structure and characterization of thymic surface antigens and their interactions with thymic humoral factors will lead to a better understanding of the regulation of T-cell immune function. The characterization of thymic peptide hormones (factors) has resulted in their potential therapeutic application to reconstitute immunodeficient patients. Therapeutic trials in progress have met with some success. The major unresolved
query to be answered by these clinical trials is whether or not the patient with cellular immune deficiency, who shows increased T-cell markers following thymic hormone treatment, does appear to be clinically improved.

**Thymic Tumors**

Primary tumors of the thymus generally occur during the third and fourth decades of life, 0.5 to 2 percent of reported cases occur in children; usually in children > 10 years of age or in adolescence. Though these neoplasms are relatively rare, they have been the subject of much study because of the wide spectrum of associated disorders involving the neuromuscular, hematologic, immunologic and endocrinologic systems. Collectively tumors of thymus are referred to as thymomas and they account for 20 percent of the tumor-like lesions of the mediastinum.

The morphologic and histologic characteristics of these neoplasms define the three major classifications of tumor. Usually they are circumscribed, encapsulated lesions varying in size up to 15 to 20 cm in diameter; more aggressive, malignant lesions penetrate the capsule. Rarely is extrathoracic spread found, and approximately one-third of the primary thymic tumors display malignant characteristics. Histologically, three general cell-patterns are found within these tumors. **Type 1**—this consists of small cells, lymphocytes, with no classical pattern and in which Hassall’s corpuscles are rare or absent. This morphologic pattern occurs in 15 percent of thymomas. **Type 2**—protoplasmic cell type is characterized by large epithelial cells with acidophilic cytoplasm occurring as islands or clusters against a lymphoid background. distinctive Hassall’s corpuscles are seen in this variety. Its incidence accounts for approximately 60 percent of thymomas. **Type 3**—This is a spindle cell pattern in which the epithelial cells histologically resemble fibroblasts. In this variant Hassall’s corpuscles are commonly observed and a vascular or lymphangiometous appearance of the tumor is frequently observed.

Only 1 percent of all thymomas recognized occur in the pediatric age group. The long term survival and the clinical characteristics of thymoma in childhood are significantly worse. The ten year survival for thymomas in adults is 60 to 70 percent whereas in several studies the survival amongst children was under two years, and systemic metastases were common.

In the context of a discussion of thymic endocrine function, two aspects of thymomas merit special discussion. It has recently been recognized that carcinoid and oat cell carcinomas of the thymus are associated with endocrine manifestations in affected patients. Carcinoid tumors may occur scattered throughout the body and present in organs particularly of epithelial nature derived from primitive digestive canal. These tumor cells have been classified as: diffuse endocrine epithelial organ, peripheral endocrine or paracrine system or amine precursors uptake and decarboxylation system (APUD). All tumors of this type are potentially malignant, and they have the capacity to invade and metastasize dependent upon their anatomic location and size. These tumors display morphologic, histochemical and behavioral properties which are a function of their site of origin. Thymic carcinoid tumors, in common with lung, stomach pancreas and duodenum, are foregut derivatives which: (a) be associated with Cushing’s syndrome or type 1 multiple endocrine adenomatisos, (b) be arranged in trabecular or gland-like structures, (c) be argyrophil, not argentaffil or (d) contain small uniformly round granules on electron microscopy.

Oat cell carcinomas of the thymus may be viewed as an undifferentiated form of carcinoid; endocrine disturbances as-
associated with this tumor are related to its hormonal secretion. This tumor contains neurosecretory cytoplasmic granules which have a high serotonin content. Twenty-four cases have been recorded in the literature. The reported patients ranged in age from 20 to 70 years, one was a boy nine years of age, and there appears to be a male preponderance. The clinical mode of presentation has varied from asymptomatic lesions, localized mediastinal symptoms and manifestations of Cushing's syndrome, multiple endocrine adenomatosis and myopathy. These tumors usually are all of low grade malignancy; however, they may metastasize to regional lymph nodes or to bone with osteoblastic lesions.

The association of thymomas with the neuromuscular disorder myasthenia gravis merits special attention. Approximately 30 percent of patients with myasthenia gravis have associated thymomas. This neurologic disorder is characterized by reduced muscle action potentials following nerve stimulation which may be corrected by treatment with cholinesterase inhibitors. It had been suggested for some time that a humoral factor binds to the post-synaptic membrane and, thus, desensitizes this region to acetyl choline. Immunological considerations of post-synaptic blocking agents have been an outgrowth of the study of the association of this neurological disease with thymoma. Approximately 90 percent of patients with myasthenia have serum anti-receptor antibodies (post-synaptic receptor). In patients with this disorder and in normals a binding-site identical to the post-synaptic muscle receptor is found in the thymus at epithelial cells in the outer part of Hassall’s corpuscles. Myasthenic patients with thymoma, following thymectomy, show clinical improvement, progressive decrease in anti-receptor antibody and a decrease in the T:B lymphocyte ratio. These data are compatible with an autoimmune nature of myasthenia gravis and are suggestive of a break in immunotolerance.

A variety of clinical situations has been associated with the occurrence of thymomas in patients. Red cell aplasia and systemic lupus erythematoses have been associated with malignant thymomas, in addition to the more commonly recognized association with myasthenia gravis. The concurrence of thymomas with these disease states has been studied too infrequently to allow any conclusions about the significance of these correlations.

Investigations on the functional significance of the thymus and its endocrine activity have opened a new vista of scientific activity. These frontiers have their scientific origins in observations from the turn of the century. They epitomize Voltaire’s views on originality. “Originality is nothing but judicious imitation—the most original writers borrowed one from another. The instruction we find in books is like fire. We fetch it from our neighbors, kindle it at home, communicate it to others, and it becomes the property of all.”

References