Clinical and Experimental Immunology REVIEW doi:10.1111/j.1365-2249.2005.03060.x

Miller's seminal studies on the role of thymus in immunity

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Accepted for publication 1 February 2006
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Summary

The thymus is one of the two primary lymphoid organs. It is responsible for the provision of T lymphocytes to the entire body, and provides a unique microenvironment in which T cell precursors (thymocytes) undergo development, differentiation and clonal expansion. This review article summarizes the seminal work of the Australian scientist Francis Albert Pierre Miller concerning the description for the first time of the crucial role of the thymus for normal development of the immune system.

Keywords: history of medicine; immunology; thymus

Introduction

The thymus was recognized as such by the Greeks. Its purpose, however, has been rendered clear in only recent times, since immunologists began to elucidate the origin and function of peripheral lymphocytes in disease.

The word itself may be derived from a Greek word, thýmos, meaning the heart or soul. The ancient Greeks used very young animals for their sacrifices. They noted a large mass of tissue in the chest above the heart that extended into the neck and concluded that it must be the seat of the soul. Galen first described the morphology of the gland and many medieval students regarded it as being at the heart of good health.

Abnormalities of the human thymus are associated with several syndromes. The first of these associations was recognized by Weigert in 1901, in his classical description of the relationship between myasthenia gravis and thymic tumours.

By the 1950s, recognition of the thymus as the site of production of lymphocytes had been well established. Their immunological competence was demonstrated unequivocally by Billingham *et al.* [1] and Gowans *et al.* [2].

Circulating lymphocytes were divided eventually into T and B cells, following the identification by Glick *et al.* [3] of the bursa of Fabricius as the source of antibody-producing cells [4]. Interest in the thymus for the generation of T cells was aroused when mammals were found to be the same in this respect.

The thymus in mouse leukaemia

In 1951, Gross injected filtered material extracted from the tissues of mice with spontaneous leukaemia into mice with low leukaemic strains which, provided newborns were injected, then developed a high incidence of leukaemia [5].

In 1958, Francis Albert Pierre Miller, after his medical degree and an internship at the Royal Prince Alfred Hospital in Sydney, received a research fellowship that enabled him to read for his PhD at the Chester Research Institute, an Institute of Cancer Research, in South Kensington, London, where Dr R. J. C. Harris suggested that he should investigate the pathogenesis of lymphocytic leukaemia in mice. This form, whether spontaneous or induced by irradiation or chemical agents, was known to involve the thymus, and adult thymectomy had prevented its development [6].

The role of the thymus in virus-induced leukaemia, on the other hand, was unknown. Miller thymectomized 4–5-week-old C3Hf/Gs mice that he had injected at birth with leukaemic extracts. None developed leukaemia [7]. Implantation of syngeneic thymus from normal mice into these thymectomized mice restored their potential for leukaemia development [8].

They were healthy until some time after weaning, when many lost weight and died prematurely, whether or not inoculated with virus. Miller thus suggested that: 'The thymus at birth may be essential for life' [9].

The thymus is essential for normal development of the immune system

Miller's neonatally thymectomized mice showed a marked deficiency of lymphocytes both in the circulation and in the lymphoid tissues. The lymphocyte/polymorph ratio did not increase significantly during the first 8 days of life and at 6 weeks of age was not much higher than at birth. Involution of the lymphoid tissues was a characteristic feature. At 6 weeks, the spleen was greatly reduced in size [10] and displayed ill-defined, inactive follicles, with little basophilia, poor cellularity and few mitoses [11,12]. There were few germinal centres. The lymph nodes were also considerably smaller and displayed inactive follicles and poor cellularity. Peyer's patches were present, but smaller and less cellular than in the controls.

Circulating lymphocytes had been shown by Billingham *et al.* [1] and Gowans *et al.* [2] to be immunologically competent and able to reject skin grafts. Miller found that thymectomized mice failed to reject skin from foreign mouse strains [12]. Female C57BL mice thymectomized at 2 weeks of age developed no immune response to syngeneic male skin grafts, but rejected allogeneic grafts [11]. Thymectomy after 3 weeks of age was unaccompanied by any significant impairment of this response. In Miller's words, therefore: 'During embryogenesis the thymus would produce the originators of immunologically competent cells, many of which would have migrated to other sites at about the time of birth. This would suggest that lymphocytes leaving the thymus are specially selected cells' [12].

The time was by no means ripe, however, for acceptance of the view that the thymus was endowed with an immune function. By contrast with the small lymphocytes taken by thoracic duct cannulation and with spleen and lymph node cells, thymocytes in general displayed little ability to initiate immune reactions after adoptive transfer. Thoracic duct lymphocytes homed from blood into lymphoid tissues, the only exception being the thymus, in which very few small lymphocytes lodge [13].

The production of antibody-forming plasma cells and the formation of germinal centres, so prominent in spleen and lymph nodes, were absent in normal thymus tissue. Defects in immune responsiveness had never been documented in mice whose thymus had been removed during adulthood, and hence it was thought that 'the thymus gland does not participate in the control of the immune response' [14]. The immune defects observed after neonatal thymectomy were soon confirmed independently by other investigators [15,16].

When mice were thymectomized 1 or more weeks after birth, i.e. when their lymphoid system and the immune mechanisms had partially developed, only negligible effects were observed.

The foreign experiments indicate that: (a) thymectomy is associated generally with a diminution in the lymphocyte population and (b) the earlier in life thymectomy is performed, the greater the deficiency of lymphocytes in other lymphoid organs. Furthermore, while diminished lymphocyte production continues in mice thymectomized during adulthood, it eventually stops in those thymectomized at birth.

Two mechanisms may account for this defect: (a) the thymus, through cell migrations, populates and continually replenishes other lymphoid tissues. This would be of major importance in very early life and decrease with age. (b) The thymus produces a non-cellular or humoral factor which regulates lymphocyte production and maturation, particularly during early life. Metcalf [17] claimed to have demonstrated in the thymus a specific lymphocytosis-stimulating-factor (LFS) whose activity was apparently associated with the epithelial–reticular cell complex of the medulla. This factor was heat-labile and filtrable, but non-dialysable.

If the immune system was destroyed in the adult, would the thymus still be involved in lymphopoiesis?

Grégoire and Duchateau [18] reported that implants of thymus tissue depleted lymphocytes by irradiation, and this comprised mainly radioresistant epithelial stroma-stimulated lymphopoiesis, whereas lymph node and muscle implants had no such effect.

Because total body irradiation destroys lymphoid tissues, Miller predicted that recovery of immune functions following irradiation would be thymus-dependent. Adult mice were thymectomized and subjected to total body irradiation. To prevent death they were given bone marrow cells, a source of haematopietic stem cells. Control, non-thymectomized mice, treated in this way recovered normal lymphoid functions within 6–8 weeks. The thymectomized mice did not [10,19].

In the adult, therefore, the thymus is still required to re-establish defence mechanisms depleted as a result of some accident or disease.

Cell transfer studies

Immune functions can be restored to animals thymectomized at birth or thymectomized and irradiated in adult life by infusing lymphocytes or implanting thymus tissue. Miller investigated the effect of injecting lymphoid cells into neonatally thymectomized mice and found the following.

1 Syngeneic thymus cells from 1-day-old mice given intravenously to newborn mice immediately after thymectomy did not prevent runting, lymphoid atrophy or immunological failure [12].
2 Syngeneic lymphoid cells from 8-week-old mice presensitized against Ak skin, on injection into 10-week-old neonatally thymectomized C3H mice carrying healthy Ak skin grafts, for more than 1 month conferred adoptive

immunity. The Ak skin was rejected within 12 days and the mice showed evidence of immunity to a second-set graft [20].

3 Allogeneic lymphoid cells from 2-month-old mice caused a severe graft-*versus*-host (GVH) reaction when injected intravenously into newborn mice immediately after thymectomy [10].

Lymphocytes restored immune capabilities, but only if the donor was syngeneic [21]. If it was of a different strain the thymectomized host wasted and died, because injected lymphocytes, being immunologically competent, reacted against the foreign tissues of their host and brought about a fatal GVH reaction.

Neonatally thymectomized mice, implanted with syngeneic thymus tissue soon after birth, developed a normal immune system. When grafted with foreign thymus tissue, they were specifically tolerant of thymus-donor type skin only [12,22].

This finding led Miller to postulate that 'When one is inducing a state of immunological tolerance in a newly born animal, one is in effect performing a selective or immunological thymectomy' [12]. In other words, the precursors of thymic lymphocytes differentiating in the presence of foreign cells and with specificities for the foreign antigens would be deleted, implying that the thymus might be the site in which self–non-self-discrimination occurs and self-tolerance is imposed.

This idea received strong support from Macfarlane Burnet, who in a lecture in June 1962 at the University of London stated: 'If, as I believe, the thymus is the site where proliferation and differentiation of lymphocytes into clones with definable immunological functions occurs, we must also endow it with another function, the elimination or inhibition of self-reactive clones' [23].

Experiments combining the techniques of thymectomy and injection of marked thymus cells led to the conclusion that thymus-derived cells were small lymphocytes, able to circulate in blood and lymph for many months in rodents and years in man [24].

The functional anatomy of the thymus

The thymus is an encapsulated gland that undergoes remarkable age-related changes. It is replenished eventually with fatty areolae that replace its normal lymphoid tissue. Disappearance of thymic structures, however, is not complete and some islands of functionally competent tissue are still recognizable in senility.

The gland displays a lobuled pattern, with distinct cortical and medullary compartments that is related strictly to its function, namely the production of fully competent circulating T cells bearing the form of the T cell receptor.

Two main cell populations are recognizable [25]. The stromal population consists of fixed ectodermal-derived, keratin-positive epithelial cells, which form a three-dimensional network occupying the cortex and the medulla. These cells are referred to comprehensively as thymic epithelial cells. The second population constitutes the parenchyma and is composed of thymocytes plus a variety of antigen-presenting cells, including interdigitating cells, macrophages and small amounts of B cells.

Thymocyte precursors reach the thymus from the blood. In the embryo they come first from the rudimentary liver and then from the bone marrow. On entering the gland they undergo proliferation, lineage commitment and selection, which is largely under the control of thymic epithelial cells.

Thymocyte positive and negative selection

Two selective processes accompany thymocyte migration, proliferation and differentiation [26]. The final result is the apoptosis of about 96% of thymocytes and only 3–5% become fully competent T cells, i.e. cells able to recognize foreign antigens, but unresponsive toward self-antigens, that eventually enter the circulation as naive T cells. Until a few years ago it was believed that negative selection occurred in the thymic cortex, whereas positive selection occurred in the medulla. Today, both compartments are thought to provide selective signals leading to cell survival or death.

Uncommitted haemopoietic progenitors therefore enter the gland through post-capillary venules at the cortical–medullary junction. They move first towards the subcapsular region and acquire T lineage commitment. Subcapsular thymocytes express both helper/inducer and suppressor/cytotoxic phenotypes ($CD4^+$ $CD8^+$ or 'double positive' thymocytes). These cells then return to the medulla and, during their passage through specific cortical zones, undergo either positive or negative selection under the guidance of both contact and paracrine signals from the epithelium [27].

Epithelial cells present thymocytes with an enormous repertoire of self-peptides conjugated to major histocompatibility complex moieties. Positive selection is obtained when these complexes on the surface of thymic cells are recognized by the T cell receptor located on the thymocyte surface. This interaction generates survival signals that rescue thymocytes from apoptosis. By contrast, negative selection occurs when such interactions are too strong or too weak, and apoptosis is promoted by the absence of survival factors. This is called 'apoptosis by neglect' [28].

Only about 4% of double-positive cells are selected positively to generate mature $CD4^+$ or $CD8^+$ cells. Once this phenotype is acquired, thymocytes enter the medulla where they remain for a few days before being released into the peripheral lymphoid pool.

The medulla and central tolerance

The structure of the medulla is substantially different from that of the cortex. Its cell population is very composite and

the stromal epithelium itself is more compact and less arborized. A close relationship has been detected recently between the architecture of the medullary stroma and the emergence of autoimmune disorders in the mouse. The correct expression of the product of the autoimmune regulator (*AIRE*) gene correlates with a normal organization of the medullary stroma [29]. By contrast, mutations in the *AIRE* gene are responsible for an autoimmune syndrome called APECED (autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy), characterized by the loss of self-tolerance to multiple organs and abnormal structure of the thymic medulla [30]. This compartment thus seems essential for instructing thymocytes to self-tolerance (central tolerance) [31].

Some 1–5% of epithelial cells in the medulla express a mosaic of 'ectopic' tissue-specific molecules, such as parathyroid hormone, thyroglobulin, insulin, C-reactive protein, etc. [32]. This has led to the formulation of the theory of 'promiscuous' gene expression, wherein the medulla represents a collection of antigenic structures that do not strictly belong to the thymus, but pertain to peripheral epithelial tissues [33].

Anatomists have long recognized that the medulla contains groups of cells displaying the organization, morphology and functional activity of other epithelial tissues, namely Hassal's bodies (squamous epithelial cells that resemble epidermal epithelium), cystic 'organoid' structures with the morphological and phenotypic features of respiratory epithelium, neuroendocrine cells, myoid cells and solitary thyroid follicles. It is thus a highly specialized structural and antigenic environment that recapitulates the spectrum of an epithelial 'self' by creating a type of 'immunological homunculus' [33].

This epithelial organization, along with its numerous antigen-presenting cells (interdigitating cells, macrophages, B cells), makes the medulla the best candidate for the accomplishment of functions such as deletion of autoreactive clones of thymocytes and induction of immunological tolerance.

Acknowledgements

Our studies were supported by Associazione Italiana per la Ricerca sul Cancro (AIRC, National and Regional Funds), Milan, the Ministry for Education, the Universities and Research (FIRB) and the Ministry for Health – Regione Puglia (grant BS2 and 'Convenzione n. 131/Ricerca Finalizzata IRCCS'), Rome, and Fondazione Italiana per la Lotta al Neuroblastoma, Genoa, Italy.

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