J. Anat. (1991), **177**, pp. 1–29
With 11 *figures*
Printed in Great Britain

Research Review

Functional anatomy of the thymic microenvironment

MARION D. KENDALL

Thymus Research Laboratory and Cell Biology Unit, United Medical and Dental Schools of Guy's and St Thomas's Hospitals, Lambeth Palace Road, London SE1 7EH, UK

INTRODUCTION

Although the thymus was recognised by the Greeks, the function of this gland has remained enigmatic until comparatively recently when the science of immunology started to elucidate the origins and functions of peripheral lymphocytes in disease. We now understand that the function of this primary lymphoid organ is to generate most if not all of the circulating T cell repertoire, and to control the production of self major histocompatibility (MHC) class II antigen-restricted lymphocytes to ensure that only lymphocytes capable of correctly distinguishing self from nonself are let loose in the body. This process is referred to as 'education', and errors in education are eliminated (negative selection) whilst the correct education process results in the cloning of these selected thymocytes (positive selection). Thymocytes are subjected both to positive and negative selection in the thymus, but these processes may well continue among postthymic T cells in the periphery. Failure to educate thymocytes correctly, or to control the release of miseducated T cells, usually results in disease, especially autoimmune conditions.

The name 'thymus' is based on one of two differently accented Greek words meaning a herb, or the heart or soul. Many mediaeval students regarded the thymus as at the heart of good health. It is reputed that Galen, who lived about 130–200 AD, first described the morphology of the gland and it has attracted sporadic interest ever since, particularly in the 1900s when hyperplasia was noted to occur in conjunction with myasthenia gravis. Thymectomies performed to ameliorate this and other conditions were reported (Veau, 1910; Schumacher & Roth, 1912). In the 1940s to 1950s Keynes pioneered the surgery of the gland in myasthenia gravis patients (Keynes, 1949) and noted that patients with thymomas prospered less well after thymectomy. This laid the foundations for the modern ideas on the recognition and treatment of thymic tumours (Keynes, 1955, 1981).

In the early 1930s, it was thought that babies and young children often suffered (and were thought to die) from an enlarged thymus (status thymicolymphaticus). In the 1930s this condition was thoroughly examined and no longer accepted as a disease entity. This period resulted in many records of thymus weight and size in fetuses, children and adults (Bratton, 1925; Greenwood & Woods, 1927; Scammon, 1927; Young & Turnbull, 1931) which have until recently formed the basis of our current understanding of thymic status in adult life. Of necessity, many of the data were taken from patients dying from terminal illnesses after long-term hospitalisation. These authors were well aware of the bias in their data, but that has been overlooked for

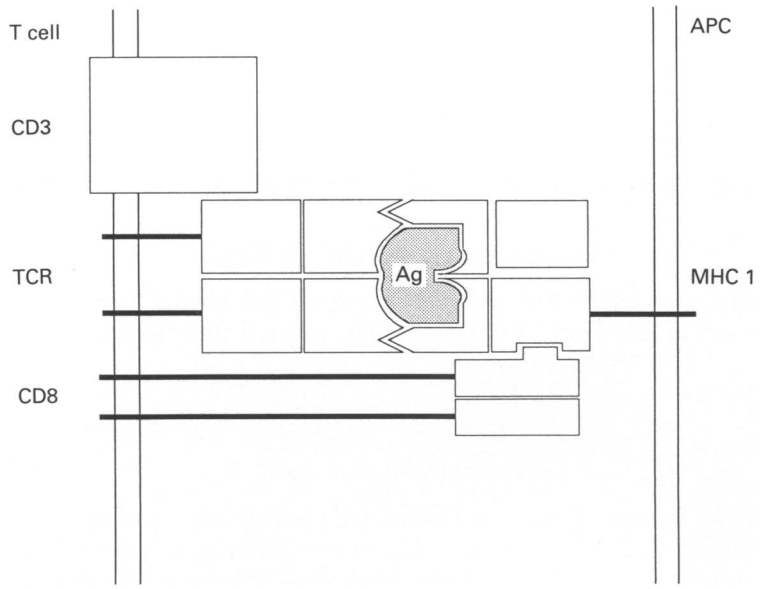

Fig. 1. A possible arrangement for the T cell receptor (TCR) and CD3 antigens on the T cell, and MHC on an antigen presenting cell (APC). The CD8 antigen interacts with MHC class I, and the foreign antigen (stippled) sits in a groove between MHC class I and the TCR.

many years, resulting in a vast underestimate of the size and activity of the adult thymus (Kendall, Johnson & Singh, 1980).

Thus the thymus was studied after disease but no specific role was attributed to it in health. Indeed, it was thought to be unnecessary. Not only could adults survive thymectomy, but the gland was often found at postmortem examination to have atrophied in adults. In neonates, however, its presence appeared vital as animals thymectomised around the time of birth died of wasting or runts' disease. More recently it has been realised that the gland also has an important role at this time in relation to the development of the sexual organs. Infants with little or no thymus at birth (e.g. Di George and cri du chat syndromes, ataxia telangectesia) all fail to develop normal gonads.

After Glick, Chang & Japp (1956) identified the bursa of Fabricius in birds as the source of antibody-producing cells, circulating lymphocytes were divided into T and B cells (Szenberg & Warner, 1962), and when mammals were found to be the same in this respect, interest in the thymus for the generation of T cells started to develop. A major problem was that T cells appeared to be devoid of any equivalent of B cell antigen receptors, immunoglobulins, but as soon as they were described, thymocytes and the thymic microenvironment became the focus of research.

Today, the structure of T cell receptors (TCR) is known in great detail (summarised in Travers, 1990; Finkel, Kubo & Cambier, 1991), and their study is an important and fertile research area. The T cell receptor (Fig. 1) is a heterodimer composed of 3 polypeptide chains (CD3) closely associated with a disulphide-linked dimer (an α and a β chain). This TCR has a groove in an exposed part of the molecule into which may fit the antigenic determinants of the major histocompatibility complex (MHC) (Salter *et al.* 1990; Wang *et al.* 1990). The T cell receptor in conjunction with CD4 surface molecules (CD4 positive T cells or T helper cells) recognises MHC class II, and in conjunction with CD8 surface molecules (CD8 positive T cells or T suppressor/

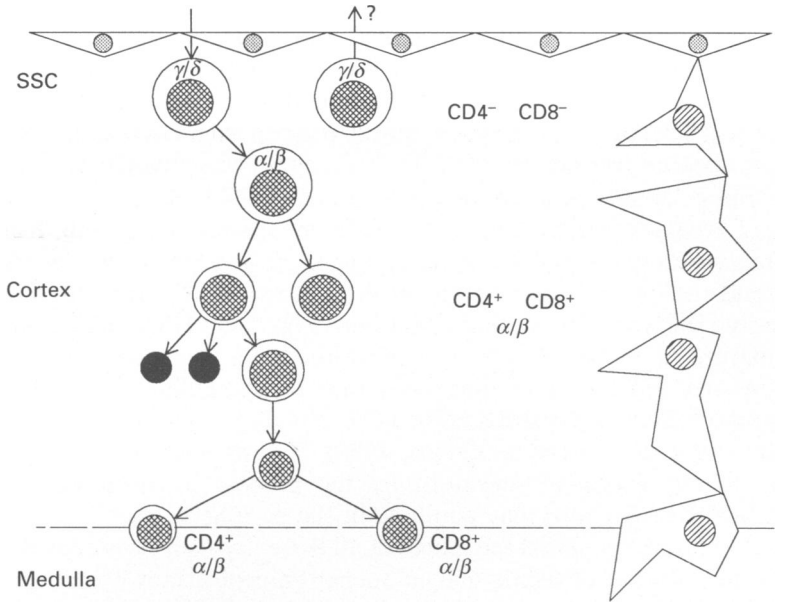

Fig. 2. A schematic and greatly simplified diagram to show the major steps in thymocyte differentiation and maturation. The top is extrathymic, and prothymocytes enter the thymus between type 1 epithelial cells possibly expressing γ/δ. Some may leave again to become peripheral γ/δ T cells. Others undergo mitosis and begin to express α/β as double negative ($CD4^-/CD8^-$) cells. These may transiently express CD4 or CD8 before becoming one of the cortical double positive ($CD4^+/CD8^+$) thymocytes. Many of these die in the thymus (●), and the others enter the medulla as single positive ($CD4^+$ or $CD8^+$) cells. Epithelial cells of the cortex (right side) provide the microenvironment for these steps.

cytotoxic cells) recognises MHC class I. Different T cells can therefore be regarded as restricted in their recognition of foreign antigen in combination with self MHC antigens. The results of either interaction in the periphery is T cell activation, e.g. the expression of factors such as interleukin-2 (Il-2) and its receptor which in turn results in proliferation and clonal expansion of the T cell.

The development of this T cell receptor complex takes place in an orderly manner mainly in the cortex of the thymus (although it may well start before prothymocytes enter the thymus) and is considered to be completed by the time the cells reach the medulla. The process of gene rearrangement results in the early expression of TCR γ/δ followed by TCR β and finally TCR α to form α/β positive cells that no longer are γ/δ positive. A population of circulating T cells, especially those associated with skin, the endothelium of the intestine and other mucosal surfaces, do however express TCRγ/δ (TCR1) (Itohara *et al.* 1990) whereas the majority of peripheral blood T cells express TCRα/β (TCR2). This has led recently to long debates as to whether there are populations of T cells that do not pass through the thymus, or do so only transiently, and whether these receptors are related to homing or specific tissue locations. It also appears that not all animals have the same proportions and intensity of expression of TCRα/β and TCRγ/δ, making the picture most complex.

Alongside the development of surface TCR, thymocytes express many other antigens, particularly those of the cluster differentiation (CD) series. This has resulted in the classification of all thymocytes into Stage I (blasts in the subcapsular region), cortical Stage II and medullary Stage III cells (Rheinherz *et al.* 1980). Such a classification has been broadly useful, but now with the recognition of many more

subtleties and subdivisions more refinement is required. In general it is considered that thymocyte precursors are 'double negative' (no CD4 or CD8 on their surface membranes), although very recent work by Wu *et al.* (1991) showed that CD4 may be expressed early and then lost as the cells become double negative (Fig. 2). The heat stable antigen (HSA) and CD2 antigen appear early in thymocyte differentiation, the Il-2 receptor appears transiently, then 60–70% of cortical thymocytes express CD8 before becoming 'double positive' (expressing both CD4 and CD8 on their surface). This series of events is briefly compared in different species in Aspinall, Kampinga & van den Bogaerde (1991). As this is occurring there is cytoplasmic development of TCR α/β and its low-level expression on the membrane. Cortical thymocytes also express binding sites for lectin peanut agglutinin (PNA) and HSA, neither of which are found on mature circulating T cells. It is most likely that negative selection occurs at this stage of development and only cells that downregulate their CD4 or CD8 expression (and HSA^+) and express more TCR α/β are positively selected to emigrate from the thymus (summarised in Crispe, 1990). The majority of cells probably die within the thymus (negative selection) by the process of apoptosis, and clonal expansion (positive selection) may continue in the periphery.

The control of developmental sequences of all these thymocytes is considered *in vivo* to be under the influence of the thymic microenvironment. Many events can influence this microenvironment. Some can be physiological changes throughout life, such as old age and pregnancy; others are infections and diseases, such as viral infections, thymomas or stress. Recently emphasis has been directed to the extreme sensitivity of the thymus for immunotoxins (see Kendall & Ritter, 1991, for review articles) and so environmental or man-made insults must also be considered. However, this paper is largely directed to reviewing our current concept of the microenvironment of the thymus and the functional implications of its anatomy.

THE NATURE OF THE THYMIC MICROENVIRONMENT

The cells of the thymic microenvironment are sessile epithelial cells and a few myoid cells, and free cells, e.g., macrophages, interdigitating cells (derived from monocytes, and therefore specialised macrophages), haemopoietic cells (eosinophils, neutrophils, B cells, plasma cells, mast cells and erythroid cells) and fibroblasts. Major blood vessels travel in connective tissue septa (outside the parenchyma of the gland) to the corticomedullary junction and there enter the cortex and medulla, to drain across the cortex to the capsule; or they travel in the medulla, back to the septa to leave alongside the arteries in the septa. Nerves reach the gland with the blood vessels, to serve them and to reach the cortex and medulla. Lymphatics form at the corticomedullary junction and leave along the connective tissue septa close to the blood vessels. Lymph nodes are often closely associated with the hilus of the gland.

Development of the thymus

In man thymic epithelium arises bilaterally from a fusion of the 3rd ventral (and probably the 4th) branchial pouch endothelium with ectoderm from the corresponding branchial clefts (Hammar, 1911; Hamilton, Boyd & Mossman, 1972; Starck, 1975). Different clefts and pouches contribute to the thymus in other animals. This interaction between the two types of epithelium appears to be essential as in nude mice embryos, the ectoderm of the 3rd cleft involutes after day $11\frac{1}{2}$, no further development of endoderm takes place and the thymus never becomes lymphoid (Cordier & Haumont, 1980).

The epithelial organ begins to attract circulating lymphoid cells that enter the rudiment before vascularisation of the organ has occurred. In avian chimeras and the mouse, waves of stem cells enter at restricted times of embryonic and possibly postnatal life (Fontaine-Perus, Culman, Kaplan & Le Douarin, 1981; Le Douarin & Jotereau, 1981). These prethymic stem cells initially originate extraembryonically from the yolk sac, then later from the fetal liver, and in neonates and adults from the bone marrow. In mice, cells capable of colonising pharyngeal pouch tissues in culture are negative for the Thy-1 thymocyte marker and are probably spleen colony forming units (CFU-S). However the degree of multipotentiality of prethymic stem cells has not been resolved, especially since different waves of immigrants (from different sources at various times throughout life) may have previously had restrictions placed on their multipotency.

As lymphoid cells enter the primordium, epithelial cells are already differentiating into subcapsular cells resting on a basal lamina and internal cells, some of which still resemble pharyngeal pouch-type cells (von Gaudecker, 1986). During 9–12 gestational weeks (g.w.) in man, septa from the mesenchyme around the primordium begin to indent the epithelial cells and create an 'opening' in the capsular surround which is advanced to the corticomedullary junction as the medulla starts to form around 14 g.w. This allows others cells, such as monocytes, to enter and become the interdigitating cells of the medulla and for nerves and blood vessels to travel in the septa to the medulla. Although blood vessels pass into the parenchyma of the gland they are always surrounded by subcapsular epithelium in the cortex and partly so in the medulla (Kendall, 1989). Medullary epithelial cells are morphologically distinct from other thymic epithelial cells and the medulla, which is fully formed by 17 g.w., contains a different population of thymocytes and accessory cells than the cortex.

The early thymic primordium is surrounded by a thin layer of mesenchyme derived from neural crest cells associated with the hindbrain. This neural crest component is important for the correct development of the thymus as extirpation experiments have shown a delayed thymic (and thyroid and parathyroid) development and abnormalities of the heart and great vessels (Bockman & Kirby, 1985). A similar range of defects occurs in several syndromes, e.g. Di George and Pierre Robin.

The neural crest also gives rise to thymic myoid cells (Nakamura & Ayer-Le Lièvre, 1986), curious muscle cells of unknown function that occur in the medulla. Certain epithelial cells may also have a neural crest origin (see below).

Eventually the left and right thymus glands come together in the midline and descend to the superior anterior mediastinum. The two glands remain discretely encapsulated by connective tissue throughout life; the blood supply, innervation and lymph drainage are separate. Generally the two glands together are erroneously referred to as one thymus.

The thymus is largest relative to body weight at birth, increases slightly in size through childhood and may be reduced in weight with age as much of the cortex is replaced with fat (Kendall *et al.* 1980; Steinmann, 1986). Thymocytes in old age have the same proliferative capabilities although their numbers are reduced, and the medulla (which is the most constant part of the gland) continues to secrete the thymic hormone, thymulin, throughout life (Kendall *et al.* 1991).

Morphological and phenotypic diversity of epithelial cells

Although many workers had described thymic epithelial cells, Wijngaert *et al.* (1984) classified them in the human thymus by their ultrastructural morphology. The classification appears to hold for all vertebrate thymus glands. Wijngaert *et al.* (1984)

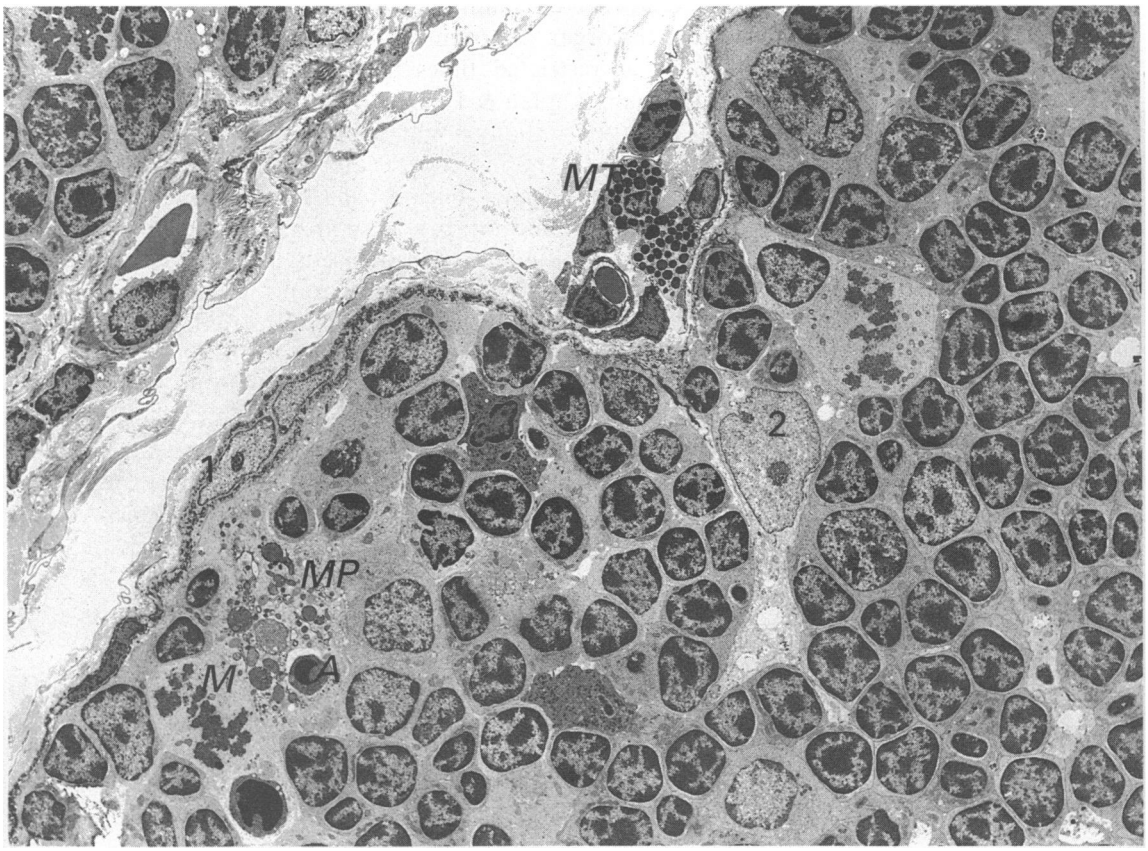

Fig. 3. Low power EM of adult rat thymus cortex. The lobule is bounded by type 1 epithelial cells (1) that are connected to type 2 cells (2) deeper in the gland. Prothymocytes (*P*) are in the subcapsular cortex where mitoses are common (*M*). Macrophages (*MP*) engulf any apoptotic cells (*A*). Two mast cells (*MT*) are adjacent to a blood capillary in the capsule. × 1500.

considered that at least 6 types (Figs 3–6) can be recognised: type 1 (subcapsular/perivascular; type 2 (cortical) which forms a series in the cortex with types 3 and 4 (electron dense, deep cortical); type 5 (medullary) and type 6 (associated with Hassall's corpuscles). Types 1 and 5 share certain cytological features, types 2 or 3 could also give rise to thymic nurse cells (TNC) which have special characteristics, type 4 cells (electron dense cells) are also found in the medulla and type 5 cells, especially in rodents, are probably composed of several subtypes with different functions.

Phenotypically the epithelial cells are not so diverse (Fig. 7), although there is a considerable degree of correspondence with morphological findings (Gaudecker *et al.* 1986; Lampert & Ritter, 1988). Many smaller differences arise when panels of antibodies are used (Kampinga *et al.* 1989; Brekelmans & van Ewijk, 1990). A more variable finding is the extent of the expression of MHC Class II (Bofill *et al.* 1985; Gaudecker *et al.* 1986). MHC antigens are probably made by epithelial cells (Rouse, Ezine & Weissman, 1985) and MHC class II expression can be induced by cytokines such as γ-interferon (γ-IFN) (Berrih *et al.* 1985; Rocha, Lehuen & Papiernik, 1988). Thus the pattern or extent of MHC class II expression could reflect changes in thymic physiology, and hence influence the nature of generated thymic subsets.

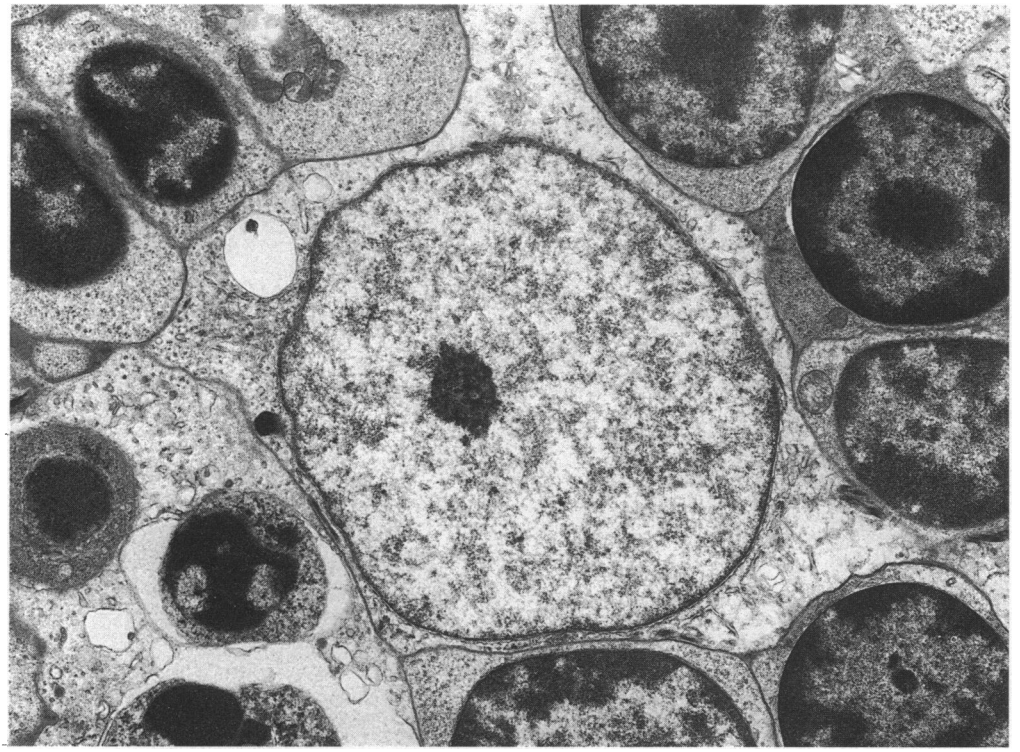

Fig. 4. Type 3 epithelial cell surrounded by cortical thymocytes. Adult rat thymus. ×7500.

One interesting and unanswered problem concerns the origin of these epithelial cells. Norris (1938) observed that in 30 mm human embryos, ectodermal cells of the cervical vesicle migrate to surround an endodermal thymus primordium completely. Gaudecker & Müller-Hermelink (1980) found that the thymus rudiments in 35 mm embryos had two morphologically distinct types of epithelial cell which were sometimes separated from each other: an outer regular row of prismatic cells and an inner mass of polygonal cells. As lymphoid cells infiltrate the organ, the distinction between different epithelial types becomes difficult to observe but Cordier & Haumont (1980) considered that the endoderm might be invaded by ectoderm in developing mouse thymuses, especially in the medulla. Much earlier, Norris (1938) had also found that as the septa invaded the epithelial primordium, so cells from the surround (which he considered to be ectodermal in origin) broke off and became part of the medulla. Although these observations were based on conventional light microscopy, it is fascinating to see that more modern immunocytochemical techniques, using MR14, MR19, RFD4, TE4 and A2B5 monoclonal antibodies and various polyclonal antibodies, show that subcapsular/perivascular epithelium and a subpopulation of medullary epithelial cells are all positive in the human thymus (Lampert & Ritter, 1988). The monoclonal antibody A2B5 recognises a complex ganglioside expressed on the cell surface of neurons, neural crest derived cells and neuropeptide secreting endocrine cells (Haynes, Shimizu & Eisenbarth, 1983). This would indicate that all subcapsular/perivascular and some medullary cells are neural ectodermal in origin. The study of Hassall's corpuscles tends to support this view as epidermis (ectodermal origin) is positive with the monoclonal antibodies MR14 and MR19 which also react

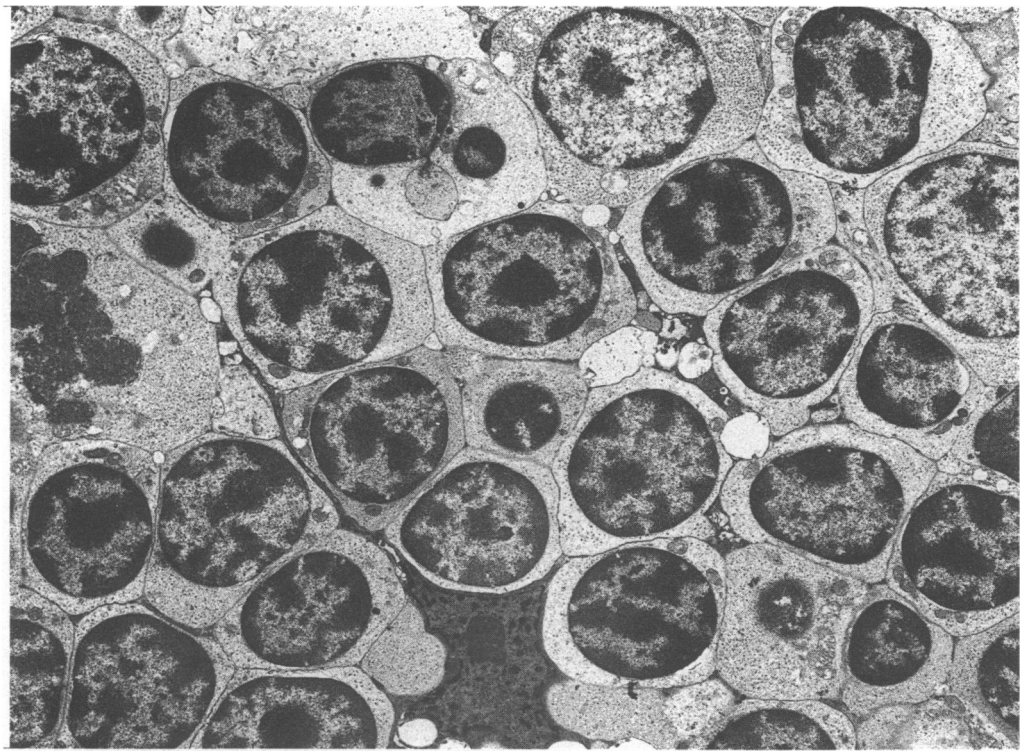

Fig. 5. The deep cortex with a type 4 epithelial cell (electron dense, lower centre) and small profiles of epithelial cell cytoplasm between most of the cells. Adult rat thymus. × 3900.

with cells around Hassall's corpuscles. Hassall's corpuscles and stratified epithelia of ectodermal origin have analogous antigens in their specific intercellular substance (Beletskaya & Gnesditskaya, 1980), as well as being positive with antibodies raised against skin keratins (Gnesditskaya & Beletskaya, 1974; Takigawa, Imamura & Ofuji, 1977).

Despite this there are problems with this interpretation of both ectodermal and endodermal components within the thymus. In the fetus (7 g.w.) the thymic rudiment is entirely epithelial and reacts throughout with A2B5, and with TE4 in the centre (Haynes, Scearce, Lobach & Hensley, 1984) although a medulla has not yet formed. It is not until much later (15 g.w.), after lymphoid cells have invaded the organ and the medulla is morphologically identifiable, that a phenotypically distinct cortex and medulla are seen. From these data and other observations concerning the induction of major histocompatibility antigen expression, their own data on mice, and R. Boyd's unpublished data on chicken, Lampert & Ritter (1988) postulated that the stem cell is positive to both cortical and subcapsular/medullary markers (a double positive cell). Immunocytochemistry of cultured rat thymic epithelial cells showed positivity for both an anticortical and an antimedullary antibody after 6–9 days. These cultures were able to form complete and functional thymus glands under the kidney capsule of nude rats (Schuurman *et al.* 1985; Kendall *et al.* 1988).

The functional importance of this arises in the study of thymomas. Although most are benign and often associated with autoimmune conditions, especially myasthenia gravis, some penetrate the thymic capsule to form metastases in surrounding tissues,

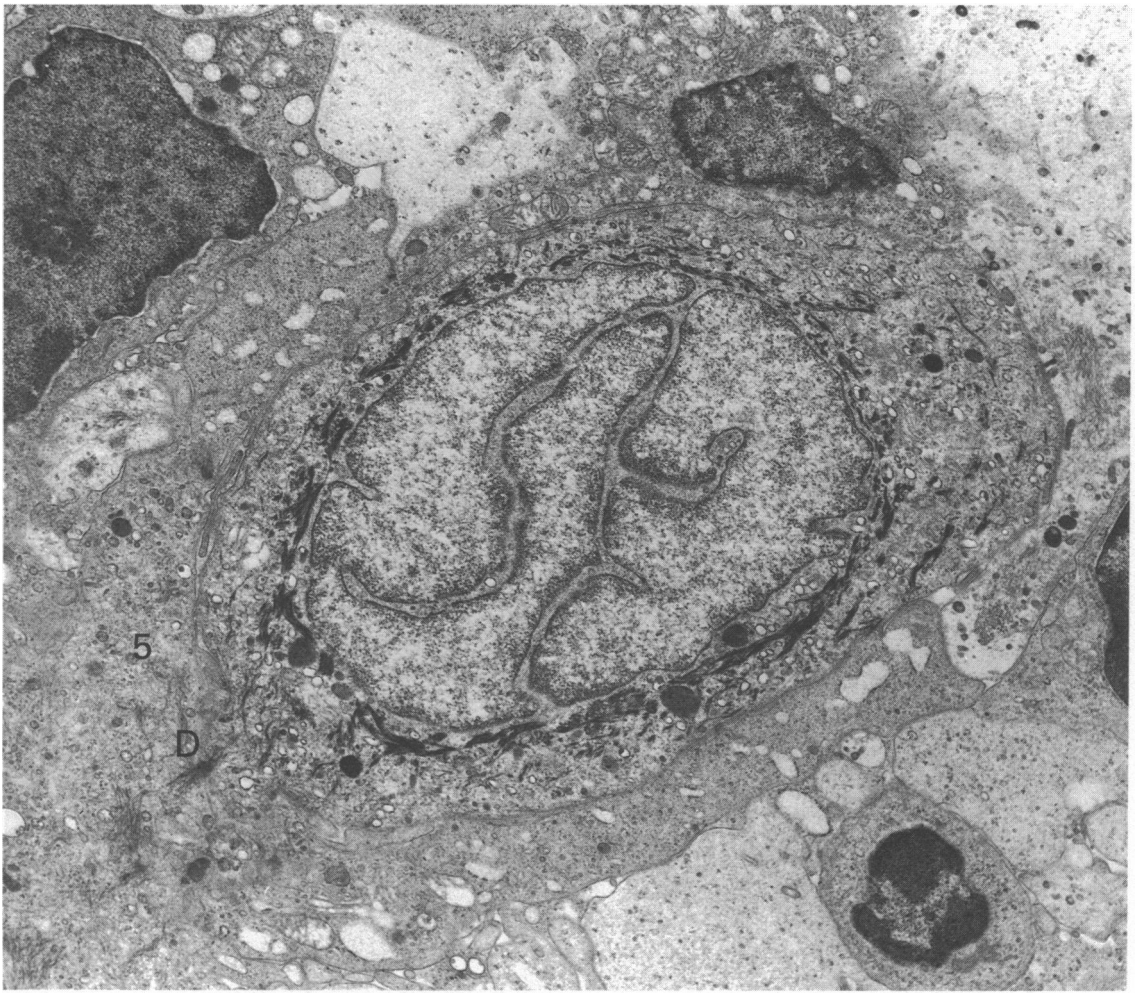

Fig. 6. An early Hassall's corpuscle (type 6 epithelial cell) in the centre joined by a desmosome (*D*) to surrounding type 5 cells (5). Adult rat thymus. × 5000.

and others are clearly malignant (Rosai & Levine, 1976). True thymomas are epithelial neoplasms that can support phenotypically normal cortical thymocytes, and those associated with myasthenia gravis (from histological criteria and single marker studies), were considered to be predominantly of cortical type (Chilosi *et al.* 1986; Müller-Hermelink, Marino & Palestro, 1986), although they do not express MHC class II antigens. However, when cortical and subcapsular/medullary markers are used together (De Maagd *et al.* 1985; Janossy *et al.* 1986; Willcox *et al.* 1987), those that were previously considered as cortical appeared to be double positive, although there is a wide spectrum of thymoma epithelial cell phenotype. There were very few purely medullary thymomas. Willcox *et al.* (1987) examined 6 invasive thymomas and 2 pleural metastases and found that 50–90 % of the epithelial cells were double positive and supported the development of cortical thymocytes. Lampert & Ritter (1988) concluded that the tumorigenic target cell is a double positive stem cell which in normal thymus development would be responsible for the entire epithelial cell

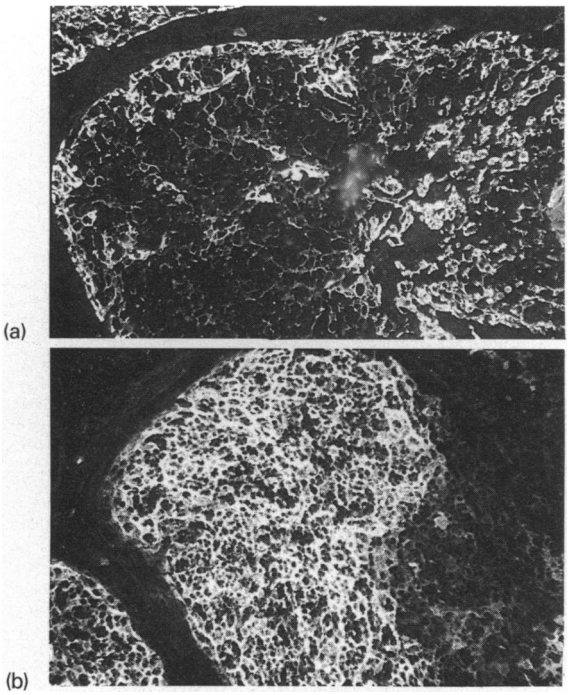

Fig. 7. Distribution of MR19 and MR6 positive cells in human paediatric thymus. (a) Subcapsular cortex and medullary epithelial cells positive with MR19. (b) Cortical epithelial cells positive with MR6. × 500.

spectrum. Whether this is ectodermal or endodermal is still not answered, although an endodermal origin would seem more plausible.

The unifying concept of the origin of thymic epithelium and the stem cells' ability to differentiate into other epithelial cell forms could explain some of the pleiomorphic appearances of thymic epithelium. Wijngaert *et al.* (1984) noted that the epithelial cells of the cortex seemed to be related in a graded morphological series which extended from the type 1 cells in the subcapsular/medullary region right across the cortex (including TNC) into some of the medullary epithelial cells. There has been a long debate amongst thymologists about the separate identity of TNCs, but with the double positive stem cell concept TNC could be the end point of one line of differentiation. Cortical types 2 or 3 cells, by emperiopoleisis of thymocytes, could become TNC. At the same time this differentiation process could also be directed to the switching on of genes for the production of molecules that appear in TNC but not most type 2 or 3 cells, e.g. oxytocin and vasopressin (Geenen *et al.* 1988).

Morphological and phenotypic diversity of thymic macrophages

Other important components of the thymic microenvironment are macrophages. It is believed that monocytes enter the gland with other circulating stem cells and differentiate there into conventional macrophages which are found at the cortico-medullary junction and in the cortex, or into interdigitating cells which typically populate the medulla (Kaiserling, Stein & Müller-Hermelink, 1974).

Macrophages are potent phagocytes, and this can be a major role of these cells in the thymus. They are especially obvious in involuting glands giving a 'starry sky'

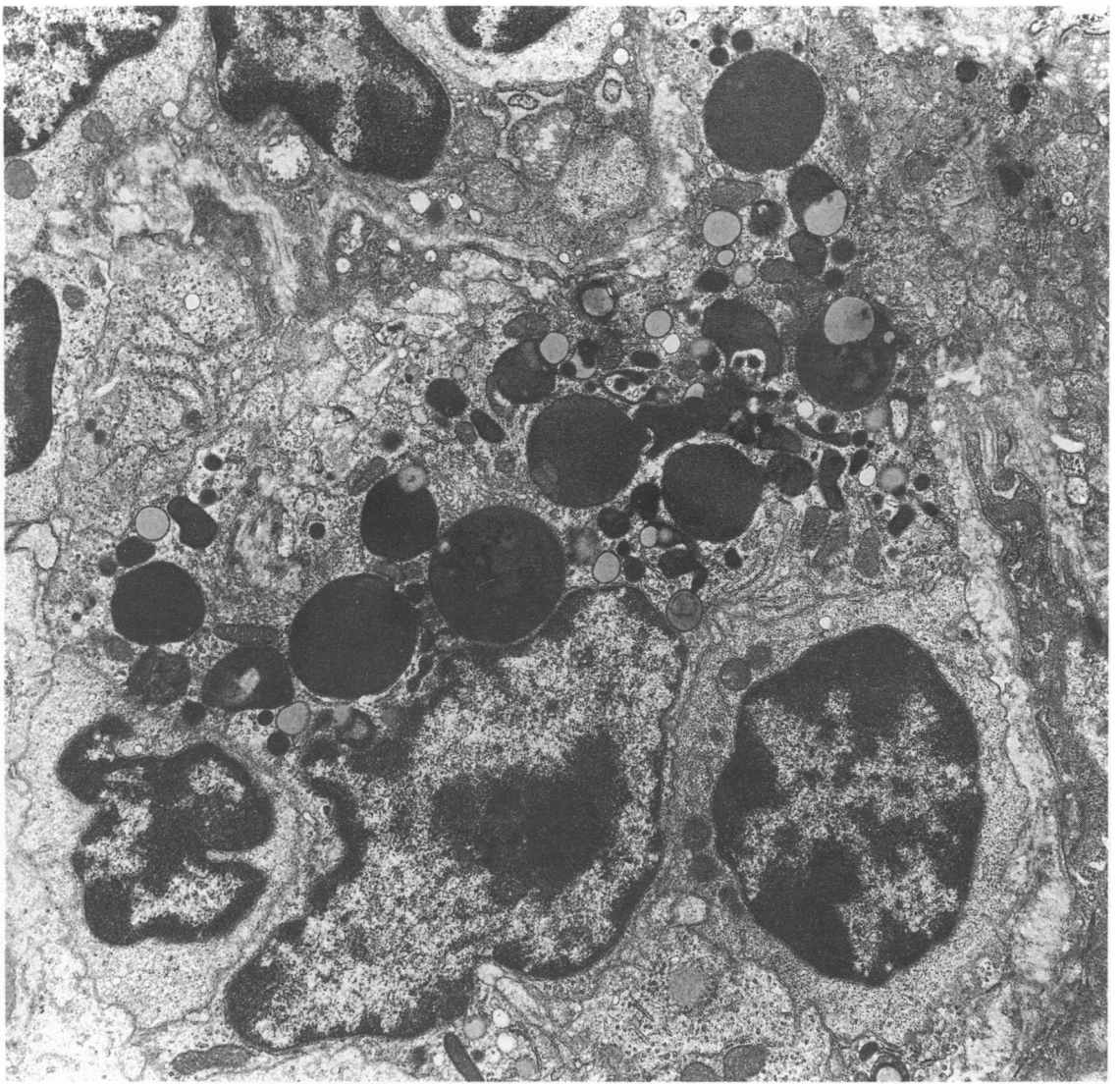

Fig. 8. A macrophage at the corticomedullary junction containing numerous remnants of apoptotic thymocyte nuclei. Adult rat thymus. × 10000.

appearance to the cortex with haematoxylin and eosin stains. At these times apoptotic thymocytes and cellular debris are found within the cytoplasm (Fig. 8). Rosettes of thymocytes around a central macrophage are commonly seen, e.g. in mouse thymus glands during pregnancy when the glands involute (Clarke & Kendall, 1990). Macrophage rosettes form tight adhesions with the thymocytes perhaps signalled by changes in thymocyte membranes that occur with the onset of apoptosis (reviewed in Kendall, 1990).

At other times phagocytosis may not be very obvious, although most macrophages usually contain a range of phagosome-like inclusions. In these situations other most important functions of macrophages are probably dominant, depending on the physiology of other cells in the local microenvironment. These functions include

cytokine production and release which probably affect all stages of thymocyte proliferation, maturation and differentiation, and finally in thymocyte ontogeny, is the specialised ability of macrophages to present antigen. Macrophages produce a large number of different cytokines and express receptors for many (e.g. IL-1, IL-2, IL-4, tumour necrosis factor-α or TNF-α_1 and γ-interferon or γ-IFN). The functional implications of this in the thymus are discussed below (p. 17).

Macrophages, as one of a number of antigen presenting cells, are central cells in immune responses. MHC class II positive macrophages take up antigen, process it and represent it in conjunction with an MHC molecule on their surfaces where T cells, particularly CD4 positive cells, are activated to proliferate (often through the release of IL-1). While it is not clear to what extent this could happen within the cortex (MHC class II positive macrophages are present there), thymic interdigitating cells are MHC class II positive. These cells have all the attributes of antigen presenting cells, and their capacity to act in this manner has recently been discussed by Hamblin & Edgeworth (1988). They concluded that tolerance induction to self-MHC and self MHC restriction are more likely to be the function of MHC class II positive cells. Indeed it is now generally accepted that negative selection can be enacted through these cells (Kyewski, Fathman & Rouse, 1986).

Entry of antigen into the thymus

Where and exactly how tolerance induction is enacted is extremely important to our concepts of immunity (see review papers in Kendall & Ritter, 1990). Thymocyte deletion in the medulla and cortex have both been experimentally demonstrated and a mechanism involving antigen-presenting cells is discussed above, but a mechanism for the cortex was more difficult to envisage despite the finding of many apoptotic cells in the cortex. The cortex in the past was generally regarded as inaccessible to circulating antigens since parenteral injection of antigen did not elicit intrathymic antibody formation. It seemed sensible that T cells in the periphery able to recognise foreign antigens would be educated in the thymus without meeting those antigens.

The maintenance of an 'antigenically pure' environment was thought to be achieved by the presence of a blood–thymic barrier (Marshall & White, 1961; Raviola & Karnovsky, 1972). Antigens had to be injected directly into the gland for a reaction to develop, and the use of electron-dense tracers showed the privileged nature of the cortex. The arrangement of blood vessels within a perivascular space itself delimited by epithelial cells of the cortex gave morphological support for this. However, this arrangement does not prevail throughout the gland (Kendall, 1989) and many papers since Sainte-Marie's early work (1963) have demonstrated that intraperitoneal injections of antigen did elicit a response from the thymus. The concepts were seriously questioned.

Recently Nieuwenhuis *et al.* (1988) demonstrated that a transcapsular route exists for many antigens to enter the thymus. This means that all developing thymocytes could be bathed by self antigens and indeed meet circulating foreign antigens. These authors have also demonstrated cortical deletion of thymocytes with a certain TCR expression following intraperitoneal injection of antibody directed to it. Kisielow & von Boehmer (1990) summarised the evidence for immature $CD4^+/CD8^+$ thymocytes expressing α/β TCR as the targets of both positive and negative selection in the thymus. Thus circulating self-antigens permeating the thymus through the transcapsular route could influence thymocyte education, resulting in tolerance induction by clonal deletion.

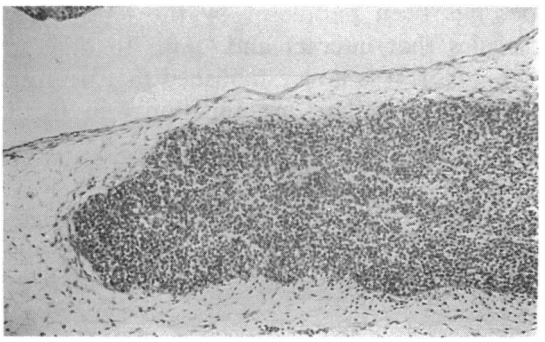

Fig. 9. Prothymocytes around and inside the thymic rudiment of a human embryo (crown–rump length 33·5 mm). Haematoxylin and eosin stain. × 80.

Entry and exit of lymphoid cells

Under the influence of chemoattractants from type 1 cells (see below), stem cells first colonise the thymus before blood vessels enter the organ (Fig. 9). Later the small numbers of cells entering make it difficult to establish entry points. Differentiation is believed to start in the subcapsular cortex, but entry of prothymocytes has been shown from large venules at the corticomedullary junction (Ceredig & Schreyer, 1984). However, it is then postulated that the cells migrate across the cortex to the subcapsular region before returning to the medulla later. It is simpler to envisage that prothymocytes could leave venules anywhere close to type 1 cells and enter the subcapsular cortex directly. This could be around the gland, along the septa or right up to the corticomedullary junction.

Monocytes, mature T cells and B cells (Abou-Rabia and M. Kendall, personal observations) also enter from the blood vessels at the corticomedullary junction, perhaps attracted by different factors. These then generally remain close to this region although mature macrophages are found throughout the cortex.

Many thymocytes become apoptotic, die *in situ* and are phagocytosed by macrophages or epithelial cells. Mature thymocytes probably leave the thymus at the corticomedullary region through high-walled postcapillary venules (not common, but a few short venules have been described) or lymphatics. The perivascular spaces of this region may be filled with lymphocytes and lymphatics originate here, deep in the gland (Kato, 1988; Kendall, 1989; Kendall *et al.* 1990). In addition, particularly in murine thymuses, cells might leave through the cystic cavities of the medulla (Khosla & Ovalle, 1986).

HOW THE THYMIC MICROENVIRONMENT FUNCTIONS

The thymus appears to be essential for the production of mature T cells, and all conditions in which the thymus is abnormal have impaired T cell responses. This function is dependent upon interactions between developing thymocytes and factors from outside the thymus (neural input, blood-borne factors), paracrine effects (intercellular interactions within the thymus) and feedback mechanisms (to the CNS and other organs). These interactions are complex and the concepts considered below are exciting growth areas in thymology. The current views may well be overthrown in future as a better understanding of neuroendocrine–immune interactions emerges.

The concept of neurotransmitters as products of neurons that interact with other

neurons or cell types has been paralleled by the term 'immunotransmitter' for immune system molecules that interact and 'talk' to other cells (Hall, McGillis, Spangelo & Goldstein, 1985). It is now established that neurotransmitters act in the immune system, immunotransmitters are functional in the CNS and numerous peptides from the hypothalamic-pituitary axis (HPA) are immunoregulatory. Of particular importance is the demonstration of the synthesis and release of pro-opiomelanocortin (POMC)-derived and growth hormone peptides from circulating lymphocytes (Blalock, Harbour-McMenamin & Smith, 1985; Weigent & Blalock, 1990) and the production of cytokines such as IL-1 by glial cells (Fontana *et al.* 1982). IL-1 is a potent activator of the HPA, especially affecting adrenocortico-trophic hormone (ACTH) and corticosterone (Dunn, 1990). While this type of event is of fundamental importance for the whole body response to antigens, CNS/thymic communications may be different and research has been directed towards defining hypothalamic–pituitary influences on the thymus, neural pathways, chemoattractants, cytokine reactions, thymic hormones, relationships with other endocrine organs and the CNS.

Hypothalamic and pituitary influences on the thymus

The hypothalamus is of prime importance in the control of the endocrine system and the autonomic nervous system. It is also structurally and functionally related to the higher regions of the nervous system and may well be the main centre through which behavioural events (e.g. those causing stress) affect the neuroendocrine–immune system. In addition the suprachiasmatic nucleus appears to be the biological clock or endogenous neural pacemaker in animals, and many factors and hormones (including those of the immune response) have circadian rhythms of release. Thus the type of immune response and its efficiency is influenced by, and affects, hypothalamic events (Besedovsky, Sorkin, Felix & Haas, 1977).

Evidence for direct interactions between the hypothalamus/pituitary axis and the thymus in chick embryos was presented when the prosencephalon and primordium of the hypophysis were removed at 33–38 h of incubation. This resulted in involution of the thymus and fewer peripheral lymphocytes (Jankovic, Isakovic & Knezevic, 1978). Thymic involution also occurred after hypophysectomy or lesions of the preoptic anterior hypothalamic area in rodents (Knutson & Lundin, 1966) and influenced immune function. Anterior pituitary growth hormone given to hypophysectomised animals produced a great increase in thymic weight (Shrewsbury & Reinhardt, 1959).

Experimental thymectomy (Pierpaoli, Bianchi & Sorkin, 1971), or the athymic state as in the hypopituitary dwarf mouse (Fabris, Pierpaoli & Sorkin, 1971), is accompanied by degranulation of the anterior pituitary growth hormone and prolactin cells (Bianchi, Pierpaoli & Sorkin, 1971). Anti-growth hormone antibodies given to intact young mice produce a wasting disease similar to that seen after thymectomy (Pierpaoli & Sorkin, 1968). The effects could be reversed by growth hormone or thyroxine treatment (Pierpaoli *et al.* 1969). Hypopituitary dwarf mice lack biological activity for thymulin at an early age (Pelletier, Montplaisir, Dardenne & Bach, 1976). Prolactin, as well as growth hormone and thyroid hormones, all increase the secretion of thymic hormones (Dardenne & Savino, 1990) *in vivo* and *in vitro*. Abnormalities of pituitary hormones in patients with hypo- and hyperthyroidism, pituitary dwarfism and acromegaly (Fabris, Mocchegiani, Pacini & Pinchera, 1986; Timsit *et al.* 1990) have all confirmed the experimental manipulations of thymulin levels reported above.

ACTH from the pituitary acts primarily on the adrenal cortex to cause the release of adrenocortical steroids, mainly cortisol which in high doses causes thymocyte death

by apoptosis. Recently, however, it has been shown that ACTH releases thymulin *in vivo* and *in vitro* (see p. 00) and this action may protect the immune system in acute stress.

Innervation

In addition to using hormonal routes, the CNS can also exert control over the thymus through neural pathways. This area of research had been greatly neglected for all the lymphoid organs until comparatively recently.

The presence of silver-stained neural profiles in the developing thymus of man was investigated by Hammar in 1935. He noted that the vagus nerve sends branches into the thymus primordia during the middle of the second month of gestation, so that by 16–20 weeks a plexus of nerves has formed in the medulla. Later, after the thymus primordia have descended, the organ acquires nerves from cervicothoracic ganglia and ansa cervicalis. Only a few papers on the thymic nerves were produced after this until the 1980s, since when there have been divergent opinions on the presence of a cholinergic innervation to the gland and several papers on the peptidergic innervation.

The demonstration of thymic innervation using retrograde tracers has been the subject of dispute since Bulloch & Moore (1981) used horseradish peroxidase (HRP). Nance, Hopkins & Bieger (1987) repeated the work with lectin-bound HRP and concluded that leakage of the label from the thymus could have accounted for the earlier results of Bulloch & Moore (1981), although Magni, Bruschi & Kasti (1987) largely confirmed them. Recently re-examination of the techniques (Tollefson & Bulloch, 1990) has confirmed that after injection of either fluorogold or HRP into the thymus, neurons in the nucleus ambiguus and retrofacial nucleus are labelled; and as in their previous study, no labelled neurons in the dorsal motor nucleus of the vagus were seen. This could indicate a possible thymic neural component of vagal visceromotor fibres.

Another approach is the use of stringently applied acetylcholinesterase enzyme histology. This shows positive profiles with blood vessels and in the medulla (Al-Shawaf, Kendall & Cowen, 1991). Some large positive cell bodies were also found in the medulla. These cells were not thymocytes, epithelial cells or myoid cells (Kendall, Al-Shawaf & Zaidi, 1989).

Since cholinergic fibres elsewhere in the body may contain vasoactive intestinal peptide (VIP) (Lundberg *et al.* 1980), we searched for VIP positivity (using a polyclonal antibody) and compared the findings with AChE positivity (Al-Shawaf *et al.* 1991). The parenchyma of the medulla has only AChE positive fibres and cells but major blood vessels have AChE and VIP positive types of fibre. Thus the thymus does appear to contain AChE positive fibres and cells but the presence of a cholinergic innervation is still not proven (Felten & Felten, 1989).

There is good evidence for a noradrenergic network of nerves in the thymus (see review by Felten & Felten, 1989). These enter the gland with blood vessels and form varicose plexuses in the subcapsular cortex and at the corticomedullary junction while individual varicose fibres penetrate into the cortex. Stimulation of sympathetic innervation and catecholamines have been shown to stimulate the egress of cells from the bone marrow, spleen and lymph nodes and a similar effect in the thymus is possible. Small clusters of terminals are found adjacent to autofluorescent cells (macrophages) and mast cells, but the functional significance of this is not known. Catecholamines may influence thymocytes *in vivo* as developing thymocytes express $beta_2$-adrenoceptors which, when stimulated *in vitro*, reduce thymocyte proliferation and enhance differentiation marker expression (Singh & Owen, 1976; Singh, 1979).

Following chemical sympathectomy with 6-hydroxydopamine (6-OHDA) at birth, thymocytes have enhanced proliferative responses (Felten *et al.* 1987).

Chemical sympathectomy has also been used to demonstrate other nerve nets in the thymus since VIP, AChE positivity and calcitonin gene related peptide (CGRP) positivity all persist after sympathectomy, while neuropeptide Y (NP-Y) reactivity (and SPG positivity) vanish. In adult rats, 6-OHDA injections caused a highly significant increase in apoptosis and decrease in cortical thymocyte numbers over an 8-day period after sympathectomy, although there was a highly significant increase in thymocyte proliferation (Kendall & Al-Shawaf, 1991). This is in sharp contrast to the drop in proliferative activity in peripheral lymphocytes in rats chemically sympathectomised at birth (Felten & Felten, 1989).

Many neuropeptides have been demonstrated in the thymus. Tachykinins and substance P (SP) occur in neural profiles (Weihe *et al.* 1989) whilst neurophysin, oxytocin (OT) and arginine vasopressin (AVP) occur in and are synthesised by subcapsular and medullary epithelium and thymic nurse cells (Geenen *et al.* 1988). AVP is mitogenic for thymocytes (Whitfield, MacManus & Gillan, 1970), whilst in the periphery both AVP and OT can replace the IL-2 requirement for γ-IFN production (Johnson & Torres, 1985). Since γ-IFN upregulates class II expression on macrophages (Basham & Merigan, 1983) amongst a variety of other actions, these neuropeptides could be involved in thymocyte development. Immunoreactive VIP was found in large lymphoid cells in the perivascular spaces, and somatostatin immunoreactivity was found in epithelial cells at the corticomedullary junction (Gomariz *et al.* 1990). Although VIP has been shown to affect lymphocyte traffic in other organs, no comparable work has been done in the thymus.

Chemoattractants

The thymus is known to attract prothymocytes, circulating stem cells or pre-T cells. One such factor, a chemotaxic peptide previously called thymotaxin, has been extensively studied (reviewed in Imhof *et al.* 1989). Substances purified from embryonic mouse thymus epithelium were a chemotaxic (m.w. 4 kDa) and a chemokinetic fraction (< 5 kDa). The chemotaxic factor (thymotaxin) with activity on haemopoietic precursors from the bone marrow was finally shown to have a m.w. of 11 kDa. This has led recently to this molecule being identified as $\beta 2$ microglobulin (the light chain of MHC Class I and CD1 molecules) (Dargemont *et al.* 1989). Cells responding to this molecule are resting cells (probably T-lineage committed), high in their expression of Thy-1, low in T and B cell differentiation markers and are cells that do not proliferate under the influence of IL-1 or IL-3 stimulation. After 3 days in culture, thymotaxin treatment resulted in significant expression of CD8 and CD4 on pre-T cells (Imhof *et al.* 1989).

Cytokines

Once prothymocytes are within the thymus cortex, they are exposed to the influences of a wide variety of secreted factors. Cytokines from thymic epithelial cells include IL-1, IL-4 and colony stimulating factor for macrophages (CSF-M); from macrophages IL-1, IL-6, TNF-α and CSF-M; and from endothelial cells IL-1, IL-6 and CSF-M. Most other known cytokines could be involved in a minor way, as the thymus can contain a wide variety of different cell types at different times. These may be specialised situations but they are nonetheless a property of the thymus (e.g. when eosinophils are found to develop in the thymus then it is likely that IL-5 is involved in this process). In addition thymocytes themselves secrete IL-1, IL-2, IL-4 and IL-6 and mature T cells also secrete γ-IFN and, when activated, TNFα.

Some principles have emerged from this bewildering array of possible interactions. In general the interactions within the thymus appear to be very specific and limited to short-range communications where cells may actually be touching (Poo, Conrad & Janeway, 1988). This has been elegantly shown at the electron microscope level by Farr, Anderson, Marrack & Kappler (1985) and von Gaudecker, Larché, Schuurman & Ritter (1989). Thus immense flexibility can be evoked depending on the repertoire of receptor expression on the responding cells. Down or up-regulation of receptor expression may also provide a neat method of controlling interactions and those dynamics can themselves be modulated by other events such as hormonal actions.

Within the thymus, the differentiation but not proliferation of early thymocytes in 14 day fetal mouse, and CD4/CD8 double negative cells in adults, is under the control of IL-7 (Henney, 1989). IL-4 is also important in the growth and differentiation of thymocytes up to 16 days of gestation in mice (Sideras *et al.* 1988) although thymocytes require a co-stimulus to respond (Zlotnik *et al.* 1987). Double negative thymocytes also proliferate *in vitro* under the influence of IL-1 (Chaudhri, Clark & Ceredig, 1988), but *in vivo* this always requires another stimulator such as a mitogen. The cytokine IL-2 (produced *in vivo* by other DN thymocytes, macrophages and mature T cells) acts on peripheral T cells to amplify clones during the immune response. Thymocytes also bear receptors for IL-2 and their expression has been shown to occur specifically during the early stages of thymocyte ontogeny on DN ($CD4^-/CD8^-$) cells. These IL-2R bearing cells show a greater propensity to enter mitosis and differentiate to mature T cells (Gearing, Wadha & Perris, 1986; Crispe, 1990) than $IL\text{-}2R^-$/DN thymocytes. The use of anti-IL-2 antibodies has been shown to block proliferation and expression of the α/β T cell receptor (Jenkinson, Kingston & Owen, 1987). Despite this, a functional role for the IL-2 receptor has been denied (Plum & De Smedt, 1988). Another cytokine with proliferative and differentiation properties is IL-6, especially in synergistic combination with IL-2 and IL-4. It may act to prepare cells for the expression of IL-2R on thymocytes, for example (Hodgkin *et al.* 1988). Both IL-2 and IL-4 may be involved in repertoire selection and expression of differentiation antigens. IL-2 induces the development of $CD3^+/\alpha/\beta TRC^+$ cells, and IL-4 enhances the expression of Thy-1 and CD45 (high molecular weight) antigen on mature postthymic T cells.

Although intrathymic events involving cytokines may be short-range, many cytokines are released under immune challenge elsewhere in the body, and at these times circulating levels of cytokines could have systemic effects that in turn may alter thymic function. One potential series of events is based on the known release of ACTH (amongst other things) by systemic administration of IL-1 (Dunn, 1990). We have recently shown that ACTH itself is a potent releaser of the thymic hormone thymulin (see below under Thymic hormones). Thymulin has been shown to induce T cell differentiation markers on prethymic cells and to influence the production of CD8 cells. This is only one possible neuroimmunoendocrine interaction, and many others are thought to act on lymphopoietic events.

Thymic hormones

The thymus produces a number of peptides which appear to act in a paracrine fashion within the thymus and also circulate in the periphery where they could act as feed-back signals to the central nervous system (CNS) (Trainin, 1974). There are 4 well defined and apparently unrelated thymic peptides which will be considered here: thymosin $\alpha 1$, thymopoietin, thymulin (facteur thymique sérique) and thymic humoral factor. Recent reviews on their biology and methods of assay detail their structure and

general characteristics (Dardenne & Bach, 1988; Safieh & Kendall, 1988) and the structure of THF has been published since then (Burstein, Buchner, Pecht & Trainin, 1988).

Thymosins

Thymosin $\alpha 1$ was isolated and purified (Low *et al.* 1979) from a complex mixture of 40–50 polypeptides called thymosin fraction 5 (TF5), and shown to be secreted by subcapsular, single or grouped epithelial cells, and cells around Hassall's corpuscles (Dalakas *et al.* 1981). Other sources of thymosin $\alpha 1$ may exist, especially in the arcuate nucleus and median eminence of the CNS (Palaszynski, Moody, O'Donohue & Goldstein, 1983) which had led to its being ascribed a role in neuroimmunomodulation (Hall, McGillis, Spangelo & Goldstein, 1985). However, it has been suggested that it is a proteolytic fragment of a larger native polypeptide, and the larger prothymosin $\alpha 1$ has been isolated from the thymus (highest concentrations), spleen, lung, kidney and brain (Haritos, Tsolas & Horecker, 1984).

Thymosin $\alpha 1$ has a wide range of immunomodulatory effects ascribed to it, including inducing the differentiation of murine cortical thymocytes modulating terminal deoxynucleotidyl-transferase (TdT) expression, stimulating lymphocyte mitogen responses, and enhancing production of interferon and macrophage migration inhibitory factor (Low & Goldstein, 1984).

Most clinical studies have used TF5 and, in general, there are significant improvements in T cell numbers and functions, decreased infections, increased weight gain and overall clinical improvement. It is nevertheless important to consider the action of the individual peptides, as for example the intraperitoneal injection of TF5, but not thymosin $\alpha 1$, causes a dose-dependent increase in serum corticosterone in rodents with normal but not elevated levels (McGillis, Hall, Vahouny & Goldstein, 1985).

Thymosin $\alpha 1$ plasma concentrations are lowered in immunodeficiency conditions and in adults with malignancy (Wara *et al.* 1982; Iwata *et al.* 1983) but raised in paediatric acquired immunodeficiency syndrome (AIDS) and AIDS-related complex (ARC) (Hersch *et al.* 1983; Rubinstein *et al.* 1986; Naylor *et al.* 1987). However, these latter observations may be erroneous as there is some sequence homology between thymosin $\alpha 1$ and proteins of different isolates of human immunodeficiency virus (HIV) (Schuurman, van Baarlen, Krone & Huber, 1988).

Very recently a thymic peptide MB-35 was purified, chemically synthesised and sequenced from TF5 (Badamchian *et al.* 1990). It has sequence homology with residues 86–120 of the nuclear histone H2A previously isolated from thymuses. It is more potent than TF5 in the stimulation of prolactin and GH release from anterior pituitary cells *in vitro*.

The amino acid sequence of thymosin $\beta 4$ (which is not an exclusive thymic hormone) has been established (Low, Hu & Goldstein, 1981). It is probably active in the early stages of thymocite differentiation as it induces the enzyme terminal deoxynucleotidyl transferase in murine thymocytes *in vitro* and *in vivo*. This thymic hormone also stimulates the secretion of luteinising hormone releasing hormone from the hypothalamus which in turn causes the release of luteinising hormone from pituitaries perfused in sequence although it does not act on the pituitary directly (Rebar, Miyake, Low & Goldstein, 1981).

Thymopoietin

Thymopoietin, like its synthetic pentapeptide thymopentin, induces early T cell differentiation and regulates the function of T cells (Goldstein *et al.* 1979; Ranges, Goldstein, Boyse & Scheid, 1982) although it was primarily recognised for its

detrimental effects on neuromuscular transmission. It is probably produced by epithelial cells of the thymus (Viamontes, Audhya & Goldstein, 1986) but ubiquitin in most body tissues, and splenin from lymph nodes and spleen, are related peptides with distinctly different biological roles. Splenin for example does not affect neuromuscular transmission, although it only differs from thymopoietin by one amino acid substitution. Both the synthetic pentapeptide thymopentin and thymopoietin bind with high affinity to the acetylcholine binding region of the acetylcholine receptor. It has been hypothesised that autoimmune thymitis in myasthenia gravis results in the hypersecretion of thympoietin with consequent impairment of acetylcholine-mediated transmission at the neuromuscular junction (Venkatasubramanian, Audhya & Goldstein, 1986). The demonstration of acetylcholine receptors in the human thymus supports the view that its origins are in the thymus (Raimond, Morel & Bach, 1984). Recently Marx reported identifying a 153 kDa molecular weight protein which shares antigenic determinants with the muscle acetylcholine receptor and is found in thymomas in myasthenia gravis patients (reported in Kendall & Ritter, 1990). Perhaps this antigen will be shown to trigger the autoimmune process in this disease.

Thymopentin has been used in animal models for autoimmunity. Lau, Freestone & Goldstein (1980) found that there was a faster loss of autoantibodies elicited by the injection of cross-reacting rat erythrocytes when thymopentin was injected than in control mice. There was also a delay in the appearance of autoantibodies when spleen cells from rat immunised mice were transferred to syngeneic recipients undergoing similar immunisation schedules.

Thymulin

Thymulin was isolated and characterised as facteur thymique sérique from porcine serum (Bach, Dardenne, Pléau & Rosa, 1976). It is a well conserved nonapeptide without species specificity that is produced by epithelial cells of the subcapsular cortex and medulla in the thymus, and circulates in the blood bound to carriers which have not yet been identified. It requires zinc for its biological activity. Thymulin binds to membrane receptors of high affinity and induces T cell markers on immature bone marrow cells and $CD3^+$ and $Lyt2^+$ positive cells in the mouse (Bach *et al.* 1971; Dardenne, Charrière & Bach, 1978). Thymulin also affects mature T cells, particularly the CD8 subset, although the effects are concentration dependent. $CD3^+$, $CD4^+$ or $CD8^+$ antigens are modulated in humans with immune deficiencies (Bordigoni *et al.* 1982).

The precise characterisation of the action of thymulin has been hampered by the lack of a good quantitiative assay. The existing bioassay has given indications that blood thymulin activity is reduced in immunodeficiencies (Incefy *et al.* 1977; Iwata *et al.* 1983; Rubinstein *et al.* 1986) and with age in healthy adults. Children with AIDS or ARC had abnormally low plasma levels before the development of peripheral blood T cell abnormalities (Rubinstein *et al.* 1986). Hypothyroidism and diabetes are also associated with low levels of thymulin activity in the serum, and treatment with the appropriate hormone restored levels to normal. Hyperthyroid patients have higher than normal levels of thymulin (Fabris & Mocchegiani, 1985). These observations and various animal endocrine manipulations suggest that thyroid hormones and insulin are both required for the continued output of thymulin and that the decreases observed in old age can be reversed.

Use of a fully quantitative, recent radioimmunoassay shows that levels are highest in neonates (2191 ± 123 fg/ml), reduced in youngsters under 20 y (1499 ± 119 fg/ml) and remain low (371 ± 18 fg/ml) for the rest of adulthood (Safieh *et al.* 1990). Preliminary work with the autoimmune condition, alopecia areata, shows that

patients have highly significantly lowered plasma thymulin levels (M. Kendall, B. Safieh and J. Fenton, unpublished).

Our current research is aimed at understanding the relationship between blood thymulin levels and thymocyte function. Preliminary results indicate that both low and high thymulin levels are associated with a low percentage of $CD8^+$ cells in the thymus which is reflected to a lesser extent in the periphery (Kendall *et al.* 1991). Thymulin appears to be a potent inhibitor of suppressor T cells in the periphery (Bach *et al.* 1981) and our studies may begin to elucidate the mechanisms involved.

This preliminary study also indicated another important facet concerning thymulin release. When thymulin levels were followed in 4 patients through surgery and into the postoperative period a consistent finding was a reduction in blood thymulin levels through surgery followed by a rise during the recovery period to levels greater than those at induction of anaesthesia. This pattern of release is close to that of cortisol and ACTH in similar patients (Chambers *et al.* 1984). This led us to measure dose-related changes in thymulin evoked by physiological levels of ACTH in incubates of thymic tissue, and by stress levels of ACTH following adrenalectomy of young rats which also removed endogenous corticosterone. There was a significant hypersecretion of thymulin with elevated serum ACTH which was mimicked by potassium and was calcium dependent (Buckingham, Safieh, Singh & Kendall, 1991; Safieh *et al.* 1991). Some peptides coreleased with ACTH have also been shown to elevate thymulin release from cultured cells (Savino *et al.* 1990). Thus ACTH, the major pituitary factor released during stress, has an important functional role in increasing the secretion of thymulin, which itself influences immune status.

Thymic humoral factor

Thymic humoral factor (THF) was characterised by Trainin & Small (1970) and used as a crude extract to restore the competence of lymphoid cells from neonatally thymectomised mice. Injection of THF protected them from wasting disease and allowed them to reject tumours and reject allogeneic skin grafts (Trainin *et al.* 1979). THF is essential for induction of clonal expansion, differentiation and maturation of T cell subsets. It also augments most T cell functions and has been used clinically to normalise CD4/CD8 ratios in severe viral infections, intensive chemotherapy in malignant disease and autoimmune conditions (Handzel *et al.* 1981; Burstein, Buchner, Pecht & Trainin, 1988; Trainin, 1988).

Hormones from other endocrine glands

There is evidence in the literature that all the major hormones released from endocrine organs can influence thymic events and/or structure (Fig. 10). Adrenal and gonadal hormones have the most potent effects, generally causing thymocyte apoptosis and reduced thymic function. Gonadectomy and adrenalectomy both increase thymus weight and this is quite striking in ageing animals (Eidinger & Garrett, 1972; Kendall *et al.* 1990). Thymocytes bear receptors for glucocorticoids, and prothymocytes are the most susceptible to glucocorticoid action with medullary thymocytes being the most resistant. The sex hormones, however, probably act through the microenvironment: specific high affinity oestrogen receptors, for example, are found on epithelial cells but not on thymocytes.

Hypertrophy of epithelial cells is seen after thyroxine treatment (Scheiff, Cordier & Haumont, 1977) although no receptors have yet been observed for the metabolite of thyroxine, T_3. Functionally this results in more thymulin-secreting cells in the gland and increased serum thymulin levels (Savino, Wolf, Aratan-Spire & Dardenne, 1984).

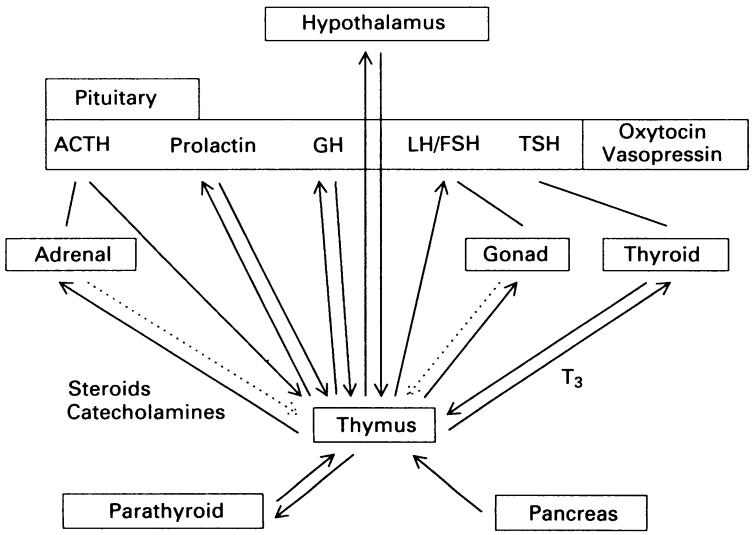

Fig. 10. Diagram of the endocrine factors that influence thymic size and function. Dotted lines indicate thymic involution. Oxytocin and vasopressin are synthesised in the thymus but their function is not known.

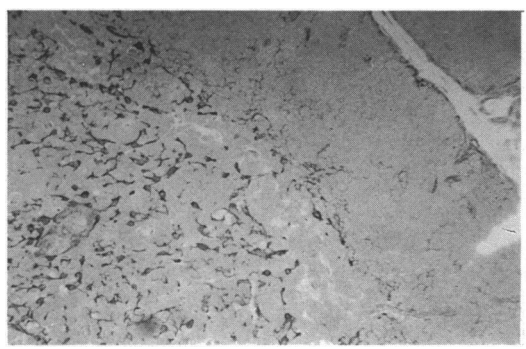

Fig. 11. Thymulin positive cells in the medulla of a human paediatric thymus. × 160.

Feedback from the thymus to the CNS

Most of the earlier work on thymic hormones as part of the bidirectional circuitry between the immune system and the CNS was undertaken by Hall and Goldstein. When it was realised that lymphocytes can synthesise POMC-derived peptides such as ACTH and opioids, and cytokines, they too were regarded as immunotransmitter agents (Hall *et al.* 1985). This attribute of lymphocytes is, however, a property of peripheral T cells and thymic hormones and nerves remain the most likely means of communication as far as the thymus is concerned.

In order to clarify these hormonal interactions, thymosins were injected directly into the CNS and peripheral hormonal changes assessed. Also studies were undertaken to localise thymosins in the CNS (Hall *et al.* 1985). TF5 injected directly into the hypothalamus resulted in increased adrenal weight, and released ACTH from cultured pituitary cells (McGillis, Hall & Goldstein, 1985). Thymosin $\alpha 1$ or $\beta 4$ caused different effects on corticosterone and LH release. LH can cause the release of thymulin. A radioimmunoassay to detect thymosin $\alpha 1$ detected it throughout the brain, especially

in the arcuate nucleus and median eminence but thymosin $\beta 4$ cross-reactivity was more ubiquitous, being found at many sites outside the brain.

Thymulin introduced by intraventricular cannulation significantly decreased hypothalamic noradrenaline levels (Vecsei, Faludi & Najbauer, 1987). Hypothalamic catecholamine concentration may also be lowered during an immune response (Roszman *et al.* 1985), and this can alter the secretion of prolactin, growth hormone and luteinising hormones as shown by chemical sympathectomy experiments (Fenske & Wuttke, 1976; Willoughby & Day, 1981). We have therefore looked in adult rats for an effect of injected thymulin (0·1–10 μg/kg) on the circulating levels of ACTH and corticosterone. There were no significant changes over a 48 h period after the injections (J. C. Buckingham, M. Kendall, B. Safieh, P. O. Cover and S. Singh, unpublished). Furthermore similar levels applied *in vitro* to either hypothalamic or pituitary incubates failed to elicit the release of corticotrophin releasing factors (CRF-41 or vasopressin), or ACTH, respectively. Thus although other thymic hormones may be important in this context, thymulin appears to have other effects.

CONCLUSIONS

The concept that the thymus in adult life is atrophied and unimportant has now to be finally rejected. Although it is not as active in adulthood as in children, it still functions to educate thymocytes before they are exported to the periphery and to secrete thymic hormones. Factors that cause thymic atrophy (e.g. abnormal hormone levels, immunotoxins), or influence its function (e.g. stress, viral infections, thymomas) can all potentially alter the nature and output of thymocytes. Such alterations could result in poor prognosis for disease. The thymus is fundamental for the maintenance of good health.

SUMMARY

This paper presents a review of our current understanding of the nature of the thymic microenvironment, after briefly considering the major role of the gland. The epithelial cells and their products are of fundamental importance, and other cells of the macrophage series are implicated in most functional events. The embryological origin of the epithelium is still not clear, although disease conditions would suggest a single origin. Immigration and emigration of thymocytes is considered, and also the passage of antigens into the gland. The events within the thymus are under the control of the CNS acting through the innervation or via hormonal pathways. Both of these areas are considered in detail, especially thymic hormone origins, functions and interactions.

The author wishes to thank her many colleagues who have contributed to her understanding of the thymus, in particular Dr Mary Ritter and Dr Henk-Jan Schuurman. She would also like to thank Amersham International, the Anatomical Society of Great Britain and Ireland and the Research (Endowments) Committee of St Thomas's Hospital for funding current research. Thanks are also due to Henk van Weering and Desmond Tobin for preparation of the figures and Dr Mary Ritter for providing Figures 7 and 11.

REFERENCES

AL-SHAWAF, A., KENDALL, M. D. & COWEN, T. (1991). Identification of neural profiles containing vasoactive intestinal polypeptides, acetylcholinesterase, and catecholamines in the rat thymus. *Journal of Anatomy* **174**, 131–143.

ASPINALL, R., KAMPINGA, J. & VAN DEN BOGAERDE, J. (1991). T-cell development in the fetus and the invariant series hypothesis. *Immunology Today* **12**, 7–10.

BACH, J.-F, DARDENNE, M., GOLDSTEIN, A. L., GUHA, A. & WHITE, A. (1971). Appearance of T-cell markers in bone marrow rosette-forming cells after incubation with thymosin, a thymic hormone. *Proceedings of the National Academy of Sciences of the USA* **68**, 2734–2738.

BACH, J.-F., DARDENNE, M., PLÉAU, J. M. & ROSA, J. (1976). Biochemical characterization of a serum thymic factor. *Nature* **266**, 55–56.

BACH, J.-F., BACH, M.-A., CHARRIÈRE, J., DARDENNE, M., ERARD, D. & RIVEAU-KAISERLIAN, D. (1981). The effect of the serum thymic factor (FTS) on suppressor T cells. In *Advances in Immunopharmacology* (ed. J. Hadden, L. Cherdid, P. Mullen & F. Spreafico), pp. 77–81. Oxford: Pergamon Press.

BADAMCHIAN, M., WANG, S.-S., SPANGELO, B. L., DAMAVANDY, T. & GOLDSTEIN, A. (1990). Chemical and biological characterization of MB-35: a thymic-derived peptide that stimulates the release of growth hormone and prolactin from rat anterior pituitary cells. *Progress in NeuroEndocrinImmunology* **3**, 258–265.

BASHAM, T. Y. & MERIGAN, T. C. (1983). Recombinant interferon-γ increases HLA-DR synthesis and expression. *Journal of Immunology* **130**, 1492–1494.

BELETSKAYA, L. V. & GNESDITSKAYA, E. V. (1980). Detection of squamous epithelial intercellular substance antigen(s) in Hassall's corpuscles of human and animal thymus. *Scandinavian Journal of Immunology* **12**, 93–98.

BERRIH, S., ARENZANA-SEISDEDOS, F., COHEN, S., DEVOS, R., CHARRON, D. & VIRELIZIER, J.-L. (1985). Interferon-γ modulates HLA Class II antigen expression on cultured human thymic epithelial cells. *Journal of Immunology* **135**, 1165–1171.

BESEDOVSKY, H., SORKIN, E., FELIX, D. & HAAS, H. (1977). Hypothalamic changes during the immune response. *European Journal of Immunology* **7**, 323–325.

BIANCHI, E., PIERPAOLI, W. & SORKIN, E. (1971). Cytological changes in the mouse anterior pituitary after neonatal thymectomy: a light and electron microscopical study. *Journal of Endocrinology* **51**, 1–6.

BLALOCK, J. E., HARBOUR-MCMENAMIN, D. & SMITH, E. M. (1985). Peptide hormones shared by the neuroendocrine and immunologic systems. *Journal of Immunology* **135**, 858s–861s.

BOCKMAN, D. E. & KIRBY, M. L. (1985). Neural crest interactions in the development of the immune system. *Journal of Immunology* **135**, 766s–768s.

BOFILL, M., JANOSSY, G., WILLCOX, N., CHILOSI, M., TREJDOSIEWICZ, M., BAGOTT, M. & NEWSOM-DAVIS, J. (1985). Microenvironments in the normal and myasthenia gravis thymus. *American Journal of Pathology* **119**, 462–473.

BORDIGONI, P., FAURE, G., BENE, M. C., DARDENNE, M., BACH, J. F., DUHEILLE, J. & OLIVE, D. (1982). Improvement of cellular immunity and IgA production in immunodeficient children after treatment with synthetic serum thymic factor (FTS). *Lancet* **ii**, 293–297.

BRATTON, A. B. (1925). The normal weight of the human thymus. *Journal of Pathology and Bacteriology* **28**, 609–620.

BREKELMANS, P. & VAN EWIJK, W. (1990). Phenotypic characterization of murine thymic microenvironments. *Seminars in Immunology* **2**, 13–24.

BUCKINGHAM, J. C., SAFIEH, B., SINGH, S. & KENDALL, M. D. (1991). Interactions of corticotrophin and glucocorticoids in the control of thymulin release in the rat. *British Journal of Pharmacology*. (In press.)

BULLOCH, K. & MOORE, R. Y. (1981). Innervation of the thymus gland by brain stem and spinal cord in the mouse and rat. *American Journal of Anatomy* **162**, 157–166.

BURSTEIN, Y., BUCHNER, V., PECHT, M. & TRAININ, N. (1988). Thymic humoral factor γ2: purification and amino acid sequence of an immunoregulatory peptide from calf thymus. *Biochemistry* **27**, 4066–4071.

CEREDIG, R. & SCHREYER, M. (1984). Immunohistological location of host and donor derived cells in the regenerating thymus of radiation bone marrow chimeras. *Thymus* **6**, 15–26.

CHAMBERS, D. J., KARIMZANDI, N., BRAIMBRIDGE, M. V., DUNHAM, J., BROOKS, F., QUINEY, J. & SLAVIN, B. (1984). Hormone and electrolyte responses during and after open heart surgery. *Thoracic and Cardiovascular Surgery* **32**, 358–364.

CHAUDHRI, G., CLARK, I. A. & CEREDIG, R. (1988). Proliferation *in vivo* of Lyt2$^-$, L3T4$^-$ thymocytes shows responsiveness to interleukin 1. *Clinical and Experimental Immunology* **73**, 51–56.

CHILOSI, M., IANNUCCI, A., FIORE-DONATI, L., TRIDENTE, G., PAMPANIN, M., PIZZOLO, G., RITTER, M., BOFILL, M. & JANOSSY, G. (1986). Myasthenia gravis: immunohistological heterogeneity in microenvironmental organization of hyperplastic and neoplastic thymuses suggesting different mechanisms of tolerance breakdown. *Journal of Neuroimmunology* **11**, 191–204.

CLARKE, A. & KENDALL, M. D. (1990). Histological changes in the mouse thymus during pregnancy. *Thymus* **14**, 65–78.

CORDIER, A. C. & HAUMONT, S. A. (1980). Development of thymus, parathyroids and ultimo-branchial bodies in NMRI and nude mice. *American Journal of Anatomy* **157**, 227–263.

CRISPE, I. N. (1990). Thymocyte precursor-product relationships: close to a consensus. *Thymus Update* **3**, 3–13.

DALAKAS, M. C., ENGEL, W. K., MCCLURE, J. E., GOLDSTEIN, A. L. & ASKANAS, V. (1981). Immunocytochemical localization of thymosin α1 in thymic epithelial cells of normal and myasthenia gravis patients and in thymic cultures. *Journal of the Neurological Sciences* **50**, 239–247.

DARDENNE, M., CHARRIÈRE, J. & BACH, J.-F. (1978). Alterations in thymocyte surface markers after *in vivo* treatment by serum thymic factor. *Cellular Immunology (New York)* **39**, 47–54.

DARDENNE, M. & BACH, J. F. (1988). Functional biology of thymic hormones. *Thymus Update* **1**, 101–116. endocrine function, cytokine expression and cell proliferation by hormones and peptides. *Progress in NeuroEndocrinImmunology* **3**, 18–25.

DARDENNE, M. & SAVINO, W. (1990). Neuroendocrine control of the thymic epithelium: modulation of thymic endocrine function, cytokine expression and cell proliferation by hormones and peptides. *Progress in NeuroEndocrinImmunology* **3**, 18–25.

DARGEMONT, C., DUNON, D., DEUGNIER, M., DENOYELLE, M., GIRAULT, J.-M., LEDERER, F., HO DIEP LE, K., GODEAU, F., THIERY, J.-P. & IMHOF, B. A. (1989). Thymotaxin, a chemotactic protein, is identical to β_2-microglobulin. *Science* **246**, 803–806.

DE MAAGD, R. A., MACKENZIE, W. A., SCHUURMAN, H.-J., RITTER, M. A., PRICE, K. M., BROEKHUISEN, R. & KATER, L. (1985). The human thymus microenvironment: heterogeneity detected by monoclonal anti-epithelial antibodies. *Immunology* **54**, 745–754.

DUNN, A. J. (1990). Interleukin-1 as a stimulator of hormone secretion. *Progress in NeuroEndocrinImmunology* **3**, 26–34.

EIDINGER, D. & GARRETT, T. J. (1972). Studies of the regulatory effects of the sex hormones on antibody formation and stem cell differentiation. *Journal of Experimental Medicine* **136**, 1098–1116.

FABRIS, N. & MOCCHEGIANI, E. (1985). Endocrine control of thymic serum factor production in young-adult and old mice. *Cellular Immunology* **91**, 325–335.

FABRIS, N., MOCCHEGIANI, E., PACINI, F. & PINCHERA, A. (1986). Thyroid function modulates thymic endocrine activity. *Journal of Clinical Endocrinology and Metabolism* **62**, 474–478.

FABRIS, N., PIERPAOLI, W. & SORKIN, E. (1971). Hormones and immunological capacity. III. The immunodeficiency disease of the hypopituitary Snell-Bagg dwarf mouse. *Clinical and Experimental Immunology* **9**, 209–225.

FARR, A. G., ANDERSON, S. K., MARRACK, P. & KAPPLER, J. (1985). Expression of antigen-specific, major histocompatibility complex-restricted receptors by cortical and medullary thymocytes *in situ*. *Cell* **43**, 543–550.

FELTEN, D. L. & FELTEN, S. Y. (1989). Innervation of the thymus. *Thymus Update* **2**, 73–88.

FELTEN, D. L., FELTEN, S. Y., BELLINGER, D. L., CARLSON, S. L., ACKERMAN, K. D., MADDEN, K. S. *et al.* (1987). Noradrenergic sympathetic neural interactions with the immune system: structure and function. *Immunological Reviews*, **100**, 225–260.

FENSKE, M. & WUTTKE, W. (1976). Effects of intraventricular 6-hyroxydopamine injections on serum prolactin and LH levels. *Brain Research* **104**, 63–70.

FINKEL, T. H., KUBO, R. T. & CAMBIER, J. C. (1991). T-cell development and transmembrane signaling: changing biological responses through an unchanging receptor. *Immunology Today* **12**, 79–85.

FONTAINE-PERUS, J. C., CULMAN, F. M., KAPLAN, C. & LE DOUARIN, N. M. (1981). Seeding of the 10-day mouse embryo thymic rudiment by lymphocyte precursors in vitro. *Journal of Immunology* **126**, 2310–2316.

FONTANA, A., KRISTENSEN, F., DUBS, R., GEMSA, D. & WEBER, E. (1982). Production of prostaglandin E and an interleukin-l-like factor by cultured astrocytes and C6 glioma cells. *Journal of Immunology* **129**, 2413–2419.

GAUDECKER, B. VON (1986). The development of the human thymus microenvironment. In *The Human Thymus. Histophysiology and Pathology* (ed. H. K. Müller-Hermelink), pp. 1–41. Berlin: Springer.

GAUDECKER, B. VON, LARCHÉ, M., SCHUURMAN, H.-J. & RITTER, M. A. (1989). Analysis of the fine distribution of thymic epithelial microenvironment molecules by immuno-electron microscopy. *Thymus* **13**, 187–194.

GAUDECKER, B. VON & MÜLLER-HERMELINK, H. K. (1980). Ontogeny and organisation of the stationary non-lymphoid cells in the human thymus. *Cell and Tissue Research* **207**, 287–306.

GAUDECKER, B. VON, STEINMANN, G. G., HANSMANN, M.-L., HARPPRECHT, J., MILICEVIC, N. M. & MÜLLER-HERMELINK, H.-K. (1986). Immunohistochemical characterization of the thymic microenvironment. A light-microscopic and ultrastructural immunocytochemical study. *Cell and Tissue Research* **244**, 403–412.

GEARING, A. J. H., WADHWA, M. & PERRIS, A. D. (1986). *In vivo* administration of interleukin 2 stimulates mitosis in thymus and bone marrow. *European Journal of Immunology* **16**, 1171–1174.

GEENEN, V., DEFRESNE, M.-P., ROBERT, F., LEGROS, J.-J., FRANCHIMONT, P. & BONIVER, J. (1988). The neurohormonal thymic microenvironment: immunocytochemical evidence that thymic nurse cells are neuroendocrine cells. *Neuroendocrinology* **47**, 365–368.

GLICK, B., CHANG, T. S. & JAPP, R. G. (1956). The bursa of Fabricius and antibody production. *Poultry Science* **35**, 224–225.

GNESDITSKAYA, E. & BELETSKAYA, L. V. (1974). Immunofluorescence study of keratin of Hassall's corpuscles and epidermis of the human skin. *Bulletin of Experimental Biology and Medicine* **77**, 431–433.

GOLDSTEIN, G., SCHEID, M. P., BOYSE, E. A., SCHLESINGER, D. H. & VAN VANWE, J. (1979). A synthetic pentapeptide with biological activity characteristic of the thymic hormone thymopoietin. *Science* **204**, 1309–1310.

GOMARIZ, R. P., LORENZO, M. J., CACICEDO, L., VINCENTE, A. & ZAPATA, A. G. (1990). Demonstration of immunoreactive vasoactive intestinal peptide (IR-VIP) and somatostatin (IR-SOM) in rat thymus. *Brain, Behaviour, and Immunity* **4**, 151–161.

GREENWOOD, M. & WOODS, H. M. (1927). 'Status thymico-lymphaticus' considered in the light of recent work on the thymus. *Journal of Hygiene (London)* **26**, 305–326.

HALL, N. R., MCGILLIS, J. P., SPANGELO, B. L. & GOLDSTEIN, A. L. (1985). Evidence that thymosins and other biological response modifiers can function as neuroactive immunotransmitters. *Journal of Immunology* **135**, 806s–811s.

HAMBLIN, A. S. & EDGEWORTH, J. D. (1888). Does antigen presentation occur in the thymus? *Thymus Update* **1**, 135–153.

HAMILTON, W. J., BOYD, J. D. & MOSSMAN, H. W. (1972). *Human Embryology*. Cambridge: Heffer; Baltimore: Williams and Wilkins.

HAMMAR, J. A. (1911). Zur grösseren Morphologie und Morphogenie der Menschenthymus. *Anatomische Heft* **43**, 201–242.

HAMMAR, J. A. (1935). Konstitutionsanatomische Studien über die Neurotisierung des Menschenembryos. *Zeitschrift für mikroskopisch-anatomie Forschung* **38**, 253–293.

HANDZEL, Z., ZAIZOV, R., VARSANO, I., LEVIN, S., PECHT, M. & TRAININ, N. (1981). In *Advances in Immunopharmacology* (ed. J. Hadden, L. Chedid, P. Muller & F. Spreafico), pp. 83–88. Oxford: Pergamon Press.

HARITOS, A. A., TSOLAS, O. & HORECKER, B. L. (1984). Distribution of prothymosin α in rat tissues. *Proceedings of the National Academy of Sciences of the USA* **81**, 1391–1395.

HAYNES, B. F., SCEARCE, R. M., LOBACH, D. F. & HENSLEY, L. L. (1984). Phenotype characterisation and ontogeny of mesodermal derived and endocrine epithelial components of the human thymic microenvironment. *Journal of Experimental Medicine* **159**, 1149–1168.

HAYNES, B. F., SHIMIZU, K. & EISENBARTH, G. S. (1983). Identification of human and rodent thymus epithelium using tetanus toxin and monoclonal antibody A2B5. *Journal of Clinical Investigation* **71**, 9–14.

HENNEY, C. S. (1989). Interleukin 7: effects on early events in lymphopoiesis. *Immunology Today* **10**, 170–173.

HERSCH, E. M., REUBEN, J. M., RIOS, A., MANSELL, P. W. A., NEWELL, G. R., MCCLURE, J. E. & GOLDSTEIN, A. L. (1983). Elevated serum thymosin α1 levels associated with evidence of immune dysregulation in male homosexuals with a history of infectious diseases or Kaposi's sarcoma. *New England Journal of Medicine* **308**, 45–46.

HODGKIN, P. D., BOND, M. W., O'GARRA, A., FRANK, G., LEE, F., COFFMAN, R. L., ZLOTNIK, A. & HOWARD, M. (1988). Identification of IL-6 as a T cell-derived factor that enhances the proliferative response of thymocytes to IL-4 and phorbol myristate acetate. *Journal of Immunology* **141**, 151–157.

IMHOF, B. A., DEUGNIER, M. A., BAUVOIS, B., DUNON, D. & THIERY, J.-P. (1989). Properties of pre-T cells and their chemotactic migration to the thymus. *Thymus Update* **2**, 3–19.

INCEFY, G. S., DARDENNE, M., PAHWA, S., GRIMES, E., PAHWA, R. N., SMITHWICK, E., O'REILLY, R. & GOOD, R. A. (1977). Thymic activity in severe combined immunodeficiency diseases. *Proceedings of the National Academy of Sciences of the USA* **74**, 1250–1253.

ITOHARA, S., FARR, A. G., LAFAILLE, J. J., BONNEVILLE, M., TAKAGAKI, Y., HAAS, W. & TONEGAWA, S. (1990). Homing of the $\gamma\delta$ thymocyte subset with homogeneous T-cell receptors to mucosal epithelia. *Nature* **343**, 754–757.

IWATA, T., INCEFY, G. S., GOOD, R. A., CUNNINGHAM-RUNDLES, S., DARDENNE, M., KAPOOR, N., KIRKPATRICK, D. & O'REILLY, R. J. (1983). Circulating thymic hormone activity in patients with primary and secondary immunodeficiency disease. *American Journal of Medicine* **71**, 385–394.

JANKOVIC, B. D., ISAKOVIC, K. & KNEZEVIC, Z. (1979). Ontogeny of the immuno-neuro-endocrine relationship. Changes in lymphoid tissue of chick embryos surgically decapitated at 33–38 hours of incubation. *Developmental and Comparative Immunology* **2**, 479–494.

JANOSSY, G., BOFILL, M., TREJDOSIEWICZ, L. K., WILLCOX, H. N. A. & CHILOSI, M. (1986). Cellular differentiation of lymphoid subpopulations and their microenvironment in the thymus. In: *The Human Thymus: Current Topics in the Microenvironment, Histophysiology and Pathology, Current Topics in Pathology, 75*. (ed. H. K. Müller-Hermelink), pp. 89–125. Berlin: Springer.

JENKINSON, E. J., KINGSTON, R. & OWEN, J. J. T. (1987). Importance of IL-2 receptors in *in vitro* thymic generation of cells expressing T receptors. *Nature* **329**, 160–162.

JOHNSON, H. M. & TORRES, B. A. (1985). Regulation of lymphokine production by arginine vasopressin and oxytocin: modulation of lymphocyte function by neurohypophyseal hormones. *Journal of Immunology* **135**, 773s–775s.

KAISERLING, E., STEIN, H. & MÜLLER-HERMELINK, H. K. (1974). Interdigitating reticulum cells in the human thymus. *Cell and Tissue Research* **155**, 47–55.

KAMPINGA, J., BERGES, S., BOYD, R. L., BREKELMANS, P., COLIC, M., VAN EWIJK, W., KENDALL, M. D., LADYMAN, H., NIEUWENHUIS, P., RITTER, M. A., SCHUURMAN, H. J. & TOURNIFIER, A. (1989). Thymic epithelial antibodies: immunohistochemical analysis and introduction of nomenclature. Summary of the Epithelium Workshop held at the 2nd Workshop 'The Thymus. Histophysiology and Dynamics in the Immune System'. *Thymus* **13**, 165–173.

KATO, S. (1988). Intralobular lymphatic vessels and their relationship to blood vessels in the mouse thymus. Light- and electron-microscopic study. *Cell and Tissue Research* **253**, 181–187.

KENDALL, M. D. (1989). The morphology of the perivascular spaces. *Thymus* **13**, 157–164.

KENDALL, M. D. (1990). The cell biology of cell death in the thymus. *Thymus Update* **3**, 47–70.

KENDALL, M. D., JOHNSON, H. R. M. & SINGH, J. (1980). The weight of the human thymus gland at necropsy. *Journal of Anatomy* **131**, 485–499.

KENDALL, M. D., SCHUURMAN, H.-J., FENTON, J., BROEKHUISEN, R. & KAMPINGA, J. (1988). Implantation of cultured thymic fragments in congenitally athymic (nude) rat. Ultrastructural characteristics of the developing microenvironment. *Cell and Tissue Research* **254**, 283–294.

KENDALL, M. D., AL-SHAWAF, A. & ZAIDI, S. A. A. (1989). The cholinergic and adrenergic innervation of the rat thymus. *Advances in Experimental Medicine and Biology* **237**, 255–261.

KENDALL, M. D., FITZPATRICK, F. T. A., GREENSTEIN, B. D., KHOYLOU, F., SAFIEH, B. & HAMBLIN, A. (1990). Changes in the thymus of rats by chemical or surgical castration. *Cell and Tissue Research* **261**, 555–564.

KENDALL, M. D. & RITTER, M. A. (1990). The role of the thymus in tolerance induction. *Thymus Update* **3**. London: Harwood Academic.

KENDALL, M. D. & AL-SHAWAF, A. (1991). The innervation of the rat thymus. *Brain, Behaviour, and Immunity*. (In press.)

KENDALL, M. D. & RITTER, M. A. (1991). The thymus in immunotoxicology. *Thymus Update* **4**. London: Harwood Academic. (In press.)

KENDALL, M. D., SAFIEH, B., SEREEN, A., MATHESON, L., VENN, G. & RITTER, M. A. (1991). Thymulin-secreting cells in man: distribution, LM histochemistry and plasma thymulin levels. In *Lymphatic Tissues and in vivo Immune Responses*. New York: Marcel Dekker. (In press.)

KEYNES, G. (1949). The results of thymectomy in myasthenia gravis. *British Medical Journal (Clinical Research)* ii, 611–616.

KEYNES, G. (1955). Investigations into thymic disease and tumour formation. *British Journal of Surgery* **42**, 449–462.

KEYNES, G. (1981). *The Gates of Memory*, pp. 273–284. Oxford: Clarendon.

KHOSLA, S. & OVALLE, W. K. (1986). Morphology and distribution of cystic cavities in the normal murine thymus. *Cell and Tissue Research* **246**, 531–542.

KISIELOW, P. & VON BOEHMER, H. (1990). Development of T cells in T cell-receptor transgenic mice: direct evidence for positive and negative selection in the thymus. *Thymus Update* **3**, 15–29.

KNUTSON, F. & LUNDIN, P. M. (1966). The effect of hypophysectomy, adrenalectomy and cortisone on the incorporation of tritiated thymidine in rat organs, with special reference to the lymphoid tissues. *Acta Endocrinologica, Copenhagen* **53**, 519–526.

KYEWSKI, B. A., FATHMAN, C. G. & ROUSE, R. V. (1986). Intrathymic presentation of circulating non-MHC antigens by medullary dendritic cells: an antigen-dependent microenvironment for T cell differentiation. *Journal of Experimental Medicine* **163**, 231–246.

LAMPERT, I. R. & RITTER, M. A. (1988). The origin of the diverse epithelial cells of the thymus: is there a common stem cell? *Thymus Update* **2**, 5–25.

LAU, C. Y., FREESTONE, J. A. & GOLDSTEIN, G. (1980). Effect of thymopoietin pentapeptide (TP5) on autoimmunity. 1. TP5 suppression of induced erythrocyte autoantibodies in C3H mice. *Journal of Immunology* **125**, 1634–1638.

LE DOUARIN, N. M. & JOTEREAU, F. V. (1981). The ontogeny of the thymus. In *The Thymus Gland* (ed. M. D. Kendall), pp. 133–155. London: Academic Press.

LOW, T. L. K., THURMAN, G. B., MCADOO, M., MCCLURE, J., ROSSIO, J. L., NAYLOR, P. N. H. & GOLDSTEIN, A. L. (1979). The chemistry and biology of thymosin. I. Isolation, characterisation and biological activities of thymosin α_1 and polypeptide α_1 from calf thymus. *Journal of Biological Chemistry* **254**, 981–986.

LOW, T. L. K., HU, S. K. & GOLDSTEIN, A. L. (1981). Complete amino acid sequence of bovine thymosin $\beta 4$: a thymic hormone that induces terminal deoxynucleotidyl transferase activity in thymocyte populations. *Proceedings of the National Academy of Sciences of the USA* **78**, 1162–1166.

LOW, T. L. K. & GOLDSTEIN, A. L. (1984). Thymosins: isolation, structural studies, and biological activities. In *The Year in Hematology* (ed. R. Silber, J. Lobuc & A. S. Gordon), pp. 21–35. New York: Plenum Press.

LUNDBERG, J., ANGGARD, A., FAHRENKRUG, J., HÖKFELT, T. & MUTT, V. (1980). Vasoactive intestinal polypeptide in the cholinergic neurones of exocrine glands: functional significance of coexisting transmitters for vasodilatation and secretion. *Proceedings of the National Academy of Sciences of the USA* **77**, 1651–1655.

MCGILLIS, J. P., HALL, N. R. & GOLDSTEIN, A. L. (1988). Thymosin fraction 5 (TF5) stimulates secretion of adrenocorticotrophic hormone (ACTH) from cultured rat pituitaries. *Life Sciences* **42**, 2259–2268.

MCGILLIS, J. P., HALL, N. R., VAHOUNY, G. V. & GOLDSTEIN, A. L. (1985). Thymosin fraction 5 causes increased serum corticosterone in rodents *in vivo*. *Journal of Immunology* **134**, 3952–3955.

MAGNI, F., BRUSCHI, F. & KASTI, M. (1987). The afferent innervation of the thymus gland in the rat. *Brain Research* **424**, 379–385.

MARSHALL, A. H. E. & WHITE, R. G. (1961). The immunological reactivity of the thymus. *British Journal of Experimental Pathology* **42**, 379–385.

MÜLLER-HERMELINK, H. K., MARINO, M. & PALESTRO, G. (1986). Pathology of thymic epithelial tumours. In *The Human Thymus. Histopathology and Pathology. Current Topics in Pathology, 75.* (ed. H. K. Müller-Hermelink), pp. 207–268. Berlin: Springer.

NAKAMURA, H. & AYER-LE LIÈVRE, C. (1986). Neural crest and thymic myoid cells. *Current Topics in Developmental Biology* **20**, 111–115.

NANCE, D. M., HOPKINS, D. A. & BIEGER, D. (1987). Re-investigation of the innervation of the thymus gland in mice and rats. *Brain, Behaviour, and Immunity* **1**, 134–147.

NAYLOR, P. H., NAYLOR, C. W., BADAMCHIAN, M., WADA, S., GOLDSTEIN, A. L., WANG, S. S., SUN, D. K., THORNTON, A. H. & SARIN, P. S. (1987). Human immunodeficiency virus contains an epitope immunoreactive with thymosin alpha 1 and the 30-amino acid synthetic p17 group-specific antigen peptide HGP-30. *Proceedings of the National Academy of Sciences of the USA* **84**, 2951–2955.

NIEUWENHUIS, P., STET, R. J. M., WAGENAAR, J. P. A., WUBBENA, A. S., KAMPINGA, J. & KARRENBELD, A.

(1988). The transcapsular route: a new way for (self)-antigens to by-pass the blood-thymus barrier? *Immunology Today* **9**, 372–375.

NORRIS, E. H. (1938). The morphogenesis and histogenesis of the thymus gland in man: in which the origin of the Hassall's corpuscles of the human thymus is discovered. *Contributions to Embryology* **27**, 191–221.

PALASZYNSKI, E. W., MOODY, T. W., O'DONOHUE, T. L. & GOLDSTEIN, A. L. (1983). Thymosin α_1-like peptides: localization and biochemical characterization in the rat brain and pituitary gland. *Peptides* **4**, 463–467.

PELLETIER, M., MONTPLAISIR, S., DARDENNE, M. & BACH, J.-F. (1976). Thymic hormone activity and spontaneous autoimmunity in dwarf mice and their littermates. *Immunology* **30**, 783–788.

PIERPAOLI, W., BARONI, C., FABRIS, N. & SORKIN, E. (1969). Hormones and the immunologic capacity. II. Reconstitution of antibody production in hormonally deficient mice by somatotropic hormone, thyrotrophic hormone and thyroxine. *Immunology* **16**, 217–230.

PIERPAOLI, W., BIANCHI, E. & SORKIN, E. (1971). Hormones and the immunological capacity. V. Modification of growth hormone-producing cells in the adenohypophysis of neonatally thymectomized germ-free mice: an electron microscopic study. *Clinical and Experimental Immunology* **9**, 889–901.

PIERPAOLI, W. & SORKIN, E. (1968). Hormones and immunological capacity. I. Effect of heterologous antigrowth hormone (ASTH) antiserum on thymus and peripheral lymphatic tissue in mice. Induction of wasting syndrome. *Journal of Immunology* **101**, 1036–1043.

PLUM, J. & DE SMEDT, M. (1988). Differentiation of thymocytes in fetal organ culture: lack of evidence for the functional role of interleukin receptor expressed by prothymocytes. *European Journal of Immunology* **18**, 795–799.

POO, W. J., CONRAD, L., JANEWAY, C. A. JR (1988). Receptor-directed focusing of lymphokine release by T helper cells. *Nature* **332**, 378–380.

RAIMOND, F., MOREL, E. & BACH, J.-F. (1984). Evidence for the presence of immunoreactive acetylcholine receptors on human thymus cells. *Journal of Neuroimmunology* **6**, 31–40.

RANGES, G. E., GOLDSTEIN, G., BOYSE, E. A. & SCHEID, M. P. (1982). T-cell development in normal and thymopoietin treated nude mice. *Journal of Experimental Medicine* **156**, 1057–1064.

RAVIOLA, E. & KARNOVSKY, M. J. (1972). Evidence for blood–thymus barrier using electron-opaque tracers. *Journal of Experimental Medicine* **136**, 466–497.

REBAR, R. W., MIYAKE, A., LOW, T. L. K. & GOLDSTEIN, A. L. (1981). Thymosin stimulates secretion of luteinizing hormone-releasing factor. *Science* **214**, 669–671.

RHEINHERTZ, E. L., KUNG, P. C., GOLDSTEIN, G., LEVEY, R. H. & SCHLOSSMAN, S. F. (1980). Discrete stages of human intrathymic differentiation: analysis of normal thymocytes and leukemic lymphoblasts of T-cell lineage. *Proceedings of the National Academy of Sciences of the USA* **77**, 1588–1592.

ROCHA, B., LEHEUN, A. & PAPIERNIK, M. (1988). IL-2 dependent proliferation of thymic accessory cells. *Journal of Immunology* **140**, 1076–1080.

ROSAI, J. & LEVINE, G. (1976). *Tumours of the Thymus*. Armed Forces Institute of Pathology Fascile: USA.

ROSZMAN, T. L., JACKSON, J. C., CROSS, R. J., TITUS, M. J. & MARKESBERRY, W. H. (1985). Neuroanatomic and neurotransmitter influences on immune function. *Journal of Immunology* **135**, 769s–772s.

ROUSE, R. V., EZINE, S. & WEISSMAN, I. L. (1985). Expression of major histocompatibility complex antigens in the thymuses of chimeric mice. *Transplantation* **40**, 422–426.

RUBINSTEIN, R., NOVICK, B. E., SICKLICK, M. J., BERNSTEIN, L. J., INCEFY, G. S., NAYLOR, P. H. & GOLDSTEIN, A. L. (1986). Circulating thymulin and thymosin-α1 activity in pediatric acquired immune deficiency syndrome: *in vivo* and *in vitro* studies. *Journal of Pediatrics* **109**, 422–427.

SAFIEH, B. & KENDALL, M. D. (1988). Methods for assaying thymic hormones. *Thymus Update* **1**, 117–133.

SAFIEH, B., KENDALL, M. D., NORMAN, J. C., MÉTREAU, E., DARDENNE, M., BACH, J. F. & PLÉAU, J. M. (1990). A new radioimmunoassay for the thymic peptide thymulin, and its application for measuring thymulin in blood samples. *Journal of Immunological Methods* **127**, 255–262.

SAFIEH, B., VENN, G., RITTER, M. A., SINGH, S., BUCKINGHAM, J. C. & KENDALL, M. D. (1991). Plasma thymulin concentrations in cardiac patients: involvement with the hypothalamic–pituitary axis. *Journal of Physiology*. (In press.)

SAINTE-MARIE, G. (1963). Antigen penetration into the thymus. *Journal of Immunology* **91**, 840–845.

SALTER, R. D., BENJAMIN, R. J., WESLEY, P. K., BUXTON, S. E., GARRETT, T. P. J., CLAYBERGER, C., KRENSKY, A. M., NORMENT, A. M., LITTMAN, D. R. & PARHAM, P. (1990). A binding site for the T-cell co-receptor CD8 on the α3-domain of HLA-A2. *Nature* **345**, 41–46.

SAVINO, W., GAGNERAULT, M.-CL., BACH, J.-F. & DARDENNE, M. (1990). Neuroendocrine control of thymic hormonal production. II. Stimulatory effects of endogenous opioids on thymulin production by cultured human and murine thymic epithelial cells. *Life Sciences* **46**, 1687–1697.

SAVINO, W., WOLF, B., ARATAN-SPIRE, S. & DARDENNE, M. (1984). Thymic hormone-containing cells. IV. Fluctuations in the thyroid hormone levels *in vivo* can modulate the secretion by human and murine epithelial cells. *Clinical and Experimental Immunology* **55**, 629–635.

SCAMMON, R. E. (1927). The prenatal growth of the human thymus. *Proceedings of the Society for Experimental Biology and Medicine* **24**, 906–909.

SCHEIFF, J. M., CORDIER, A. C. & HAUMONT, S. (1977). Epithelial cell proliferation in thymic hyperplasia induced by triiodothyronine. *Clinical and Experimental Immunology* **27**, 516–521.

SCHUMACHER, E. D. & ROTH, J. (1912). Thymektomie bei einem Fall von Morbus Basedowi mit Myasthenie. *Mitteilungen Grenzgebeiten der Medizin und Chirurgie (Jena)* **25**, 746–765.

SCHUURMAN, H.-J., VOS, J. G., BROEKHUISEN, R., BRANDT, C. J. W. M. & KATER, L. (1985). *In vivo* biological effect of allogeneic cultured thymic epithelium on thymus-dependent immunity in athymic nude rats. *Scandinavian Journal of Immunology* **21**, 21–30.

SCHUURMAN, H.-J., BAARLEM, J. VAN, KRONE, W. J. A. & HUBER, J. (1988). The thymus in the acquired immune deficiency syndrome. *Thymus Update* **1**, 171–189.

SHREWSBURY, M. M. & REINHARDT, W. O. (1959). Effect of pituitary growth on lymphatic tissues, thoracic duct lymph flow, lymph protein and lymphocyte output in the rat. *Endocrinology* **65**, 858–860.

SIDERAS, P., FUNA, K., ZALCBERG-QUINTANA, I., XANTHOPOULOS, K. G., KISIELOW, P. & PALACIOS, R. (1988). Analysis by *in situ* hybridization of cells expressing mRNA for interleukin 4 in the developing thymus and in peripheral lymphocytes from mice. *Proceedings of the National Academy of Sciences of the USA* **85**, 218–221.

SINGH, U. (1979). Effect of catecholamines on lymphopoiesis in fetal mouse thymic explants. *Journal of Anatomy* **129**, 279–292.

SINGH, U. & OWEN, J. J. T. (1976). Studies on the maturation of thymus stem cells – the effects of catecholamines, histamine, and peptide hormones on the expression of T alloantigens. *European Journal of Immunology* **6**, 59–62.

STARCK, D. (1975). *Embryologie, ein Lehrbuch auf Allgemein Biologischer Grundlage*, 3rd edn. Stuttgart: Thieme.

STEINMANN, G. G. (1986). Changes of the human thymus during ageing. In *The Human Thymus. Histophysiology and Pathology. Current Topics in Pathology, 75* (ed. H. K. Müller-Hermelink), pp. 43–88. Berlin: Springer.

SZENBERG, A. & WARNER, N. L. (1962). Immunological functions of thymus and bursa of Fabricius. Disassociation of immunological responsiveness in fowls with a hormonally arrested development of lymphoid tissues. *Nature* **194**, 146–147.

TAKIGAWA, M., IMAMURA, S. & OFUJI, S. (1977). Demonstration of epidermis-specific heteroantigens in thymic epithelial cell. *International Archives of Allergy and Applied Immunology* **55**, 58–60.

TIMSIT, J., SAFIEH, B., GAGNERAULT, M.-C., SAVINO, W., LUBETZKI, J., BACH, J.-F. & DARDENNE, M. (1990). Augmentation des taux circulantes de thymuline au cours de l'hyperprolactinémie et de l'acromégalie. *Compte Rendu de l'Académie des Sciences (Paris)* **31**, III, 7–13.

TOLLEFSON, L. & BULLOCH, K. (1990). Dual-label retrograde transport: CNS innervation of the mouse thymus distinct from other mediastinum viscera. *Journal of Neuroscience Research* **25**, 20–28.

TRAININ, N. (1974). Thymic hormones and the immune response. *Physiological Reviews* **54**, 272–315.

TRAININ, N. (1988). Therapeutic prospects of THF – a thymic hormone – in autoimmune processes. *Israel Journal of Medical Sciences* **24**, 739–740.

TRAININ, N. & SMALL, M. (1970). Studies on some physicochemical properties of a thymus humoral factor conferring immunocompetence on lymphoid cells. *Journal of Experimental Medicine* **132**, 885–889.

TRAININ, N., ROTTER, V., YAKIR, Y., LEVE, R., HANDZEL, Z., SHOHAT, B. & ZAIZOV, R. (1979). Biochemical and biological properties of THF in animal and human models. *Annals of the New York Academy of Sciences* **332**, 9–22.

TRAVERS, P. (1990). One hand clapping. *Nature* **348**, 393–394.

VEAU, V. (1910). Rapport sur la chirurgie du thymus. *Compte Rendu de l'Association Française de Pédiatrie* **1**, 77–126.

VECSEI, L., FALUDI, M. & NAJBAUER, J. (1987). The effect of 'Facteur thymique sérique' (FTS) on catecholamine and serotonin neurotransmission in discrete brain regions of mice. *Acta Physiologica Hungarica* **69**, 129–132.

VENKATASUBRAMANIAN, K., AUDHYA, T. & GOLDSTEIN, G. (1986). Binding of thymopoietin to the acetylcholine receptor. *Proceedings of the National Academy of Sciences of the USA* **83**, 3171–3174.

VIAMONTES, G. I., AUDHYA, T. & GOLDSTEIN, G. (1986). Immunohistochemical localization of thymopoietin with an antiserum to synthetic cys-thymopoietin$_{28-39}$. *Cellular Immunology* **100**, 305–313.

WANG, J., YAN, Y., GARRET, T. P. J., LIU, J., RODGERS, D. W., GARLICK, R. L., TARR, G. E., HUSAIN, Y., REINHERTZ, E. L. & HARRISON, S. C. (1990). Atomic structure of a fragment of human CD4 containing two immunoglobulin-like domains. *Nature* **348**, 411–418.

WARA, D. W., MARTIN, N. L., WARA, W. M. *et al.* (1982). Thymosin-alpha 1 levels in children with primary cellular immunodeficiency and in adults with malignancy. In *Current Concepts in Human Immunology and Cancer Immunomodulation* B. Serrou *et al.* New York: Biomedical Press.

WEIGENT, D. & BLALOCK, J. E. (1990). Growth hormone and the immune system. *Progress in NeuroEndocrineImmunology* **3**, 231–241.

WEIHE, E., MULLER, S., FINK, T. & ZENTEL, H. J. (1989). Tachykinins, calcitonin gene-related peptide and neuropeptide Y in nerves of the mammalian thymus: interactions with mast cells in autonomic and sensory neuroimmunomodulation? *Neuroscience Letters* **100**, 77–82.

WHITFIELD, J. F., MACMANUS, J. P. & GILLAN, D. J. (1970). The possible mediation by cyclic AMP of the stimulation of thymocyte proliferation by vasopressin and the inhibition of this mitogenic action by thyrocalcitonin. *Journal of Cellular Physiology* **76**, 65–76.

WIJNGAERT, F. P. VAN DE, KENDALL, M. D., SCHUURMAN, H.-J., RADEMAKERS, L. H. M. P. & KATER, L.

(1984). Heterogeneity of human thymus epithelial cells at the ultrastructural level. *Cell and Tissue Research* **237**, 227–237.

WILLCOX, N., SCHLEUP, M., RITTER, M. A., SCHUURMAN, H.-J., NEWSOM-DAVIS, J. & CHRISTENSSON, B. (1987). Myasthenic and non-myasthenic thymoma. An expansion of a minor cortical epithelial subset? *American Journal of Pathology* **127**, 447–460.

WILLOUGHBY, J. O. & DAY, T. A. (1981). Central catecholamine depletion: effects of physiological growth hormone and prolactin secretion. *Neuroendocrinology* **32**, 65–69.

WU, L., SCOLLAY, R., EGERTON, M., PEARSE, M., SPANGRUDE, G. J. & SHORTMAN, K. (1991). CD4 expressed on earliest T-lineage precursor cells in the adult murine thymus. *Nature* **349**, 71–74.

YOUNG, M. & TURNBULL, H. M. (1931). An analysis of the data collected by the status lymphaticus investigation committee. *Journal of Pathology and Bacteriology* **34**, 213–258.

ZLOTNIK, A., RANSOM, J., FRANK, G., FISCHER, M. & HOWARD, M. (1987). Interleukin 4 is a growth factor for activated thymocytes: possible role in T cell ontogeny. *Proceedings of the National Academy of Sciences of the USA* **84**, 3856–3860.