Histol Histopathol (1995) 10: 761-778

Histology and Histopathology

Invited Review

The relevance of cell microenvironments for the appearance of lympho-haemopoietic tissues in primitive vertebrates

A.G. Zapata, M. Torroba, A. Vicente, A. Varas, R. Sacedón and E. Jiménez
Department of Cell Biology, Faculty of Biology, Complutense University, 28040 Madrid, Spain

Summary. In higher vertebrates, mainly in mammals, a role for the non-lymphoid components of lymphoid organs in governing the maturation and functioning of immune system has been largely demonstrated. In contrast, such a role in the evolution of the vertebrate immune system has only been evidenced indirectly. In the present review we summarize histophysiological results which emphasize the relevance of lympho-haemopoietic stromal elements in the emergence and evolution of vertebrate lymphoid organs. The most primitive vertebrates, the Agnatha, have no true lymphoid organs and, accordingly, their immune responses seem more related to the non-anticipatory defence mechanisms of invertebrates than to the immune responses of vertebrates. So, the appearance and evolution of vertebrate lymphoid organs seems closely related with the emergence of immune capacities. Thymus, spleen and gut-associated lymphoid organs appear early in phylogeny whereas lymph nodes and bone marrow are late phylogenetical adquisitions. However, bone marrow-less vertebrates contain numerous organs (i.e., gonads, kidney, brain, etc...), the cell micro-environments of which support lympho-haemopoiesis mimicking the condition of higher vertebrate bone marrow. On the other hand, the lack of germinal centres, another feature of the lymphoid organs of ectothermic vertebrates which impedes the selection of B cells raised after somatic hypermutation, presumably reflects the absence of some of the elements necessary for this organization.

Key words: Vertebrates, Thymus, Spleen, Lymph nodes, Bone marrow, GALT

Offprint requests to: Prof. Agustín G. Zapata, Department of Cell Biology, Complutense University, 28040 Madrid, Spain

Introduction

Twenty years ago the pattern of occurrence of the lymphoid organs of lower vertebrates was established on the basis of similar knowledge in mammals (Good et al., 1966; Le Douarin, 1966; Fichtelius et al., 1968; Pontius and Ambrosius, 1971; Cooper, 1976a; Fange, 1982; Manning and Horton, 1982; Muthukkaruppan et al., 1982). Apart from these pioneer studies, and a limited amount of electron microscope data describing the ultrastructure of such organs in a few species (see review by Zapata and Cooper, 1990), little is known about the phylogenetic and functional significance of the lymphoid tissue emerging from the most primitive vertebrates. The lack of reagents, principally monoclonal antibodies raised specifically against the surface cell markers of these primitive animals and, in many cases, difficulties of manipulating them in laboratory conditions have impeded advancement in this field of Comparative Immunology. In the present article, we trace, from a histo-physiological perspective, the emergence and evolutionary trends of both the central and peripheral lymphoid organs of vertebrates, in an attempt to establish phylogenetic correlations between lymphoid organs in the most primitive vertebrates and in mammals, emphasizing the relevance of non-lymphoid cell microenvironments in the process.

A clear separation between haemopoietic, central and peripheral lymphoid organs cannot be sustained in all vertebrates

In mammals, the lymphoid organs can be classified into three categories (Picker and Siegelman, 1993):

- Lympho-haemopoietic organs, including the yolk sac, the intraembryonic mesenchyme, the foetal liver and the bone marrow. In these, pluripotent stem cells home and differentiate in each blood cell lineage.

- Primary central lymphoid organs comprising of the thymus and the bone marrow, as a main source of mammalian B lymphocytes. In this case, cell progenitors, whose abilities to produce non-lymphoid cells is still a matter of discussion, differentiate either to T or B lymphocytes in an epithelial cell micro-environment. On the other hand, in primary organs, lymphoid maturation is independent of antigen stimulation.

- Secondary peripheral lymphoid organs, including the spleen, the lymph nodes, and the lymphoid tissue associated either with the gut or respiratory tract, which are colonized by mature lymphocytes coming from the central lymphoid organs early in postnatal life. The lymphoid cells proliferate there after antigenic stimulation.

In lower vertebrates these three categories of lymphoid organs cannot always be clearly identified; thus they are generally referred to as lympho-haemopoietic tissues. Moreover, the most primitive vertebrates, the Agnatha, have no true lymphoid organs and must therefore be considered as a special case (Fange and Zapata, 1985; Zapata and Cooper, 1990).

The thymus appears for the first time in Chondrichthyes and shows the same histological organization throughout vertebrate phylogeny (Zapata and Cooper, 1990). The lack of a clear cortex-medulla demarcation in the thymus of teleosts and some primitive amphibians, and of Hassall's bodies in ectothermic vertebrates, and especially its remarkable condition in adult teleosts retaining the embryonic connection to the pharyngeal cavity (Fig. 1), are just small variations from the common pattern which do not really reflect a lack of functional capabilities.

The bursa of Fabricius is a lymphoid organ exclusive to most birds which is concerned with generation of the B cell repertoire rather than with maturation of the B cell system (Toivanen et al., 1987; Weill and Reynaud, 1987).

Bone marrow involved functionally in lympho-haemopoiesis appears for the first time in the most evolved Urodela of the Plethodontidae family (Curtis et al., 1979). Remarkably in bone marrowless vertebrates many different organs assume the functional role of bone marrow in homing and differentiating lympho-haemopoietic cell progenitors (Fig. 2).

The spleen, like the thymus, also appears in chondrichthyes and remains largely unchanged in all vertebrates (Zapata and Cooper, 1990). Nevertheless, the amount of splenic lymphoid tissue varies largely in distinct vertebrate classes reflecting the pattern of splenic blood circulation and/or the occurrence of other peripheral lymphoid organs which trap and process antigens rather than building up specific immune responses. For example, the teleost, the kidney of which is an important lymphoid organ with a capacity for trapping and responding to antigens, have poorly developed splenic lymphoid tissue (Fig. 3). In contrast, the spleen is an important lymphoid organ in adult elasmobranches but does not contain renal lymphoid tissue (Fig. 4).

Isolated lymphoid cells occur in the gut of all vertebrates, including Agnatha (Fig. 5), but true lymphoid aggregates only appear in the gut lamina propria from the Chondrichthye level, although these do not reach the category of isolated lymphoid organs until the higher vertebrates with the appearance of tonsils and Peyer's patches (Fig. 6) (Zapata and Cooper, 1990). Nevertheless, a mucosal immune system presumably occurs in all gnathostomata (Lobb and Clem, 1981; Tomonaga et al., 1986; Hart et al., 1988; Du Pasquier, 1993a).

The lymph nodes appear late in phylogeny, occurring only in birds and mammals, although some lymphoid aggregates associated with veins or lymphatic vessels present in some amphibians and reptiles have been claimed as possible phylogenetic precursors (Kotani, 1959; Kent et al., 1964; Kampmeier, 1969; Johnston, 1973). Furthermore, lymphoid organs which exclusively occur in the anurans of the Ranidae family, such as the jugular bodies (Fig. 7), have also been identified as primitive lymph nodes (Cooper, 1976b; Manning and Horton, 1982). Although these do not morphologically resemble mammalian lymph nodes they are highly efficient in eliciting immune responses against lymph-born antigens (Villena and Zapata, 1981; Zapata et al., 1981b).

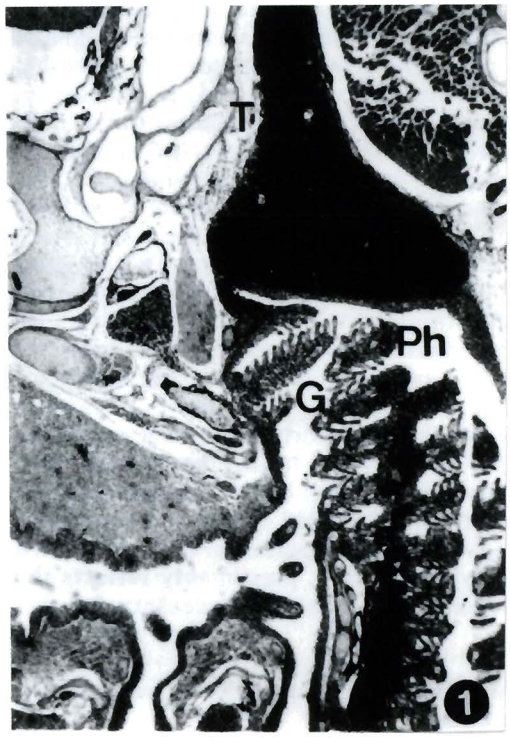


Fig. 1. Thymus (T) of an adult teleost. Note its connection with the pharyngeal cavity (Ph) and the lack of a cortex-medulla demarcation. Gills (G). x 36

Bone marrowless vertebrates contain numerous lympho-haemapoietic organs morphophysiologically equivalent to the bone marrow of higher vertebrates

One of the most remarkable aspects of the immune system of lower vertebrates, principally in primitive fish, is the occurrence of numerous lympho-haemopoietic organs which share structural and functional resemblances to the bone marrow of higher vertebrates (Fig. 2). Apparently, in these bone marrowless vertebrates any organ provided with a stromal cell microenvironment histophysiologically similar to that which supports the haemopoietic activity in bone marrow can home and differentiate blood cell progenitors. Therefore, as we discuss later, if these inductive lympho-haemopoietic cell microenvironments change structurally during ontogeny and/or life cycle, the organ can lose its lympho-haemopoietic capacities.

In bone marrow of higher vertebrates the stromal cell components are comprised of fibroblastic reticular cells, adventitial cells, macrophages, fat cells and blood sinusoidal endothelia (Metcalf, 1992). In the lympho-haemopoietic organs of lower vertebrates there is the same histological organization. For example, most elasmobranches contain masses of lympho-haemopoietic tissue in the esophagus and gonad walls, constituting the so-called Leydig and epigonal organs, respectively

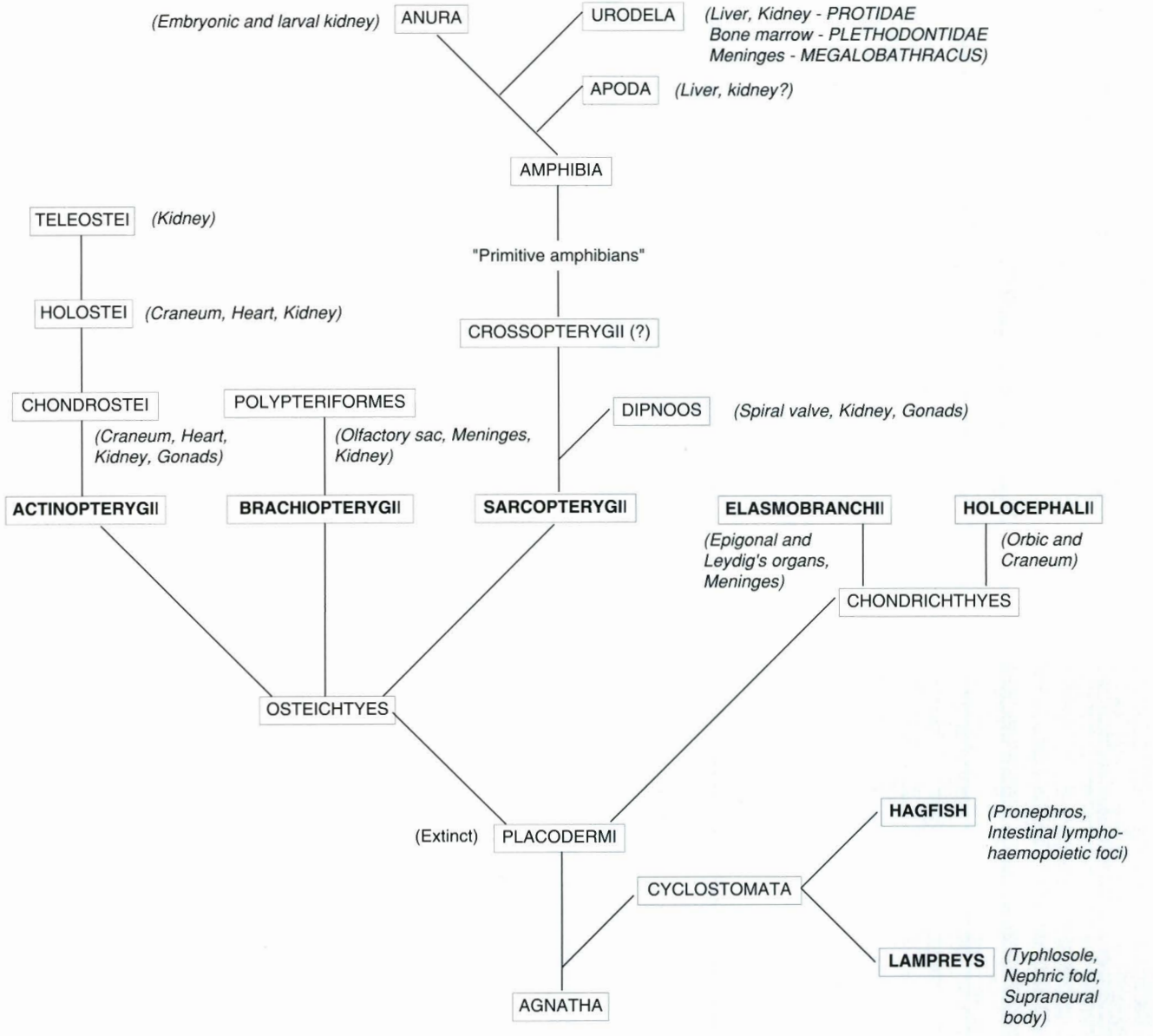


FIG. 2. Bone marrow equivalents in primitive vertebrates.

(Fange, 1982, 1984; Zapata and Cooper, 1990). In both loci, the lympho-haemopoietic tissue occupies cell cords arranged among blood sinusoids (Fig. 8). The cell cords consist of a supporting stroma of reticular cell processes and poorly developed collagen fibres which house developing and mature granulocytes, lymphocytes and plasma cells (Figs. 8, 9). In the case of the kidney, which homes lympho-haemopoietic tissue in many lower vertebrates, including Cyclostomes, Chondrostei, Holostei, Dipnoi, Polypteriformi, Teleostei, some Urodelands and embryonic Anurans (Zapata and Cooper, 1990), the cell cords appear arranged among the renal tubules and enlarged blood sinusoids (Fig. 10), which exhibit phagocytic capacity and permit migration of mature blood cells (Fig. 11).

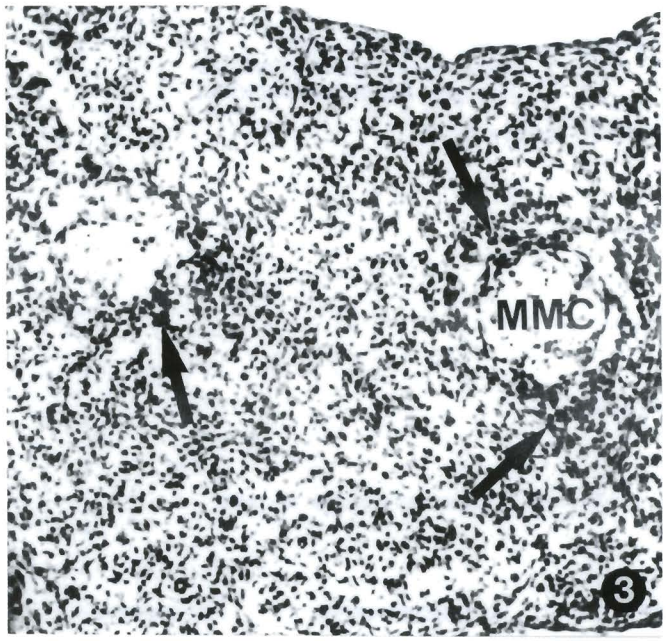


Fig. 3. Spleen of a teleost showing the poor development of lymphoid tissue (arrows) arranged around the melano-macrophage centres (MMC). x 80

The brain and the cranium also represent suitable organs for homing and differentiation of lympho-haemopoietic cell precursors in some primitive vertebrates. Several years ago it was proposed that certain extinct Devonian fishes had a meningeal lympho-haemopoietic tissue (Bjerring, 1984; Fange, 1984). We and other authors have demonstrated the occurrence of such tissues in the meninx primitiva of some elasmobranches (Chiba et al., 1988) and in the orbita and subcranium of the Holocephali, *Chimera monstrosa* (Mattisson and Fange, 1986; Mattisson et al., 1990). This tissue is presumably analogous to that occurring in the meninges of ganoids (Chandler, 1911; van der Horst, 1925; Tilney, 1927; Vialli, 1932; Scharrer, 1944) and in the brain of the urodelans *Ambystoma* (Dempster, 1930) and *Megalobatrachus japonicus* (Sano and Imai, 1961), it also occurs throughout the central nervous system, although it is more frequent in telencephalon, diencephalon, including pituitary gland, and mesencephalon. It consists of lympho-haemopoietic cells, including developing and mature granulocytes, lymphocytes, plasma cells, lymphoblasts and macrophages, closely associated to reticular cells and masses of collagen fibres (Fig. 12).

Menyngeal lympho-haemopoietic tissue of the elasmobranch central nervous system is not only involved in blood cell production. Recent data suggest that it is also a source of macrophages, lymphocytes and plasma cells which can gain access to brain ventricles forming macrophage-lymphocyte cell clusters, presumably in response to pathogens present in the cerebrospinal fluid (Torroba et al., 1995). In fact, we accidentally found macrophage-lymphocyte cell clusters in the hypothalamic ventricle of some specimens of different elasmobranch species, including the smooth dogfish *Triakis scyllia*, and the cloudy dogfish *Scyliorhinus torazame*. Serial sectioning of the hypothalamus demonstrated the existence of lymphoid tissue

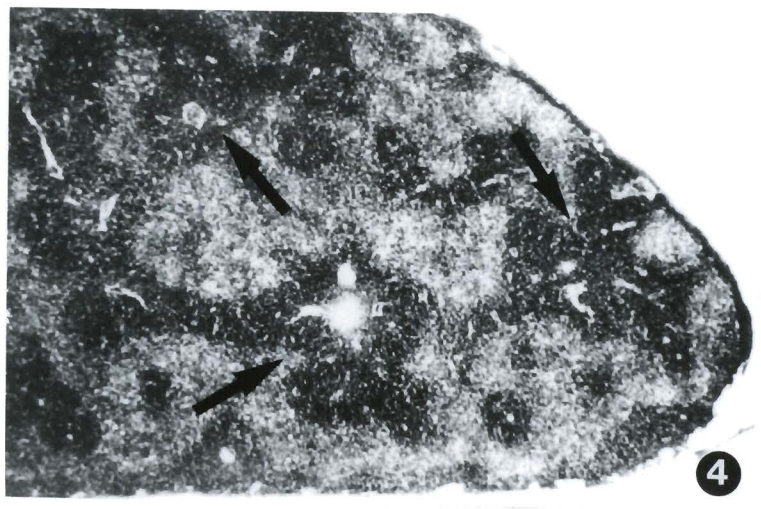


Fig. 4. Large masses of lymphoid tissue (arrows) occur around big blood vessels in the spleen of elasmobranches. x 12

which extended from the meningeal lympho-haemopoietic tissues along large blood vessels to the lumen of the third ventricle. The distinct cell components of this tissue however never migrate, into the nervous tissue because both a continuous basement membrane and a layer of glial cell processes are interposed between both tissues. Nevertheless, some macrophages can be occasionally seen to cross the ependymal cell layer (Fig. 13) into the ventrical lumen, forming cell clusters there with differently sized lymphocytes and plasma cells (Fig. 14) which resemble those described during immune response in both mammals (Nielsen et al., 1974) and other elasmobranch lymphoid organs (Zapata, 1980; Pulsford et al., 1982). In these cell clusters, typical antigen-presenting cells establish close, interdigitating, surface contacts with the neighbouring lymphocytes (Fig. 15). In other species of elasmobranches also examined, such as the gummy shark, *Mustelus manazo*, morphologically similar cell aggregates occur in the lumen of the third ventricle but do not reach the peripheral lympho-haemopoietic tissue of the meninge.

Changes in the cell microenvironments determine the locus of the lympho-haemopoietic tissues in primitive vertebrates

As mentioned previously, the kidney is one of the most common organs in which lympho-haemopoiesis occurs in lower vertebrates (Fig. 2). Furthermore, its condition during ontogeny in some vertebrate groups clearly illustrates the capacity of haemopoietic cell microenvironments to determine the functions of these organs. Remarkably, in amphibians which do not have haemopoietic bone marrow, the kidney retains its lympho-haemopoietic function. Also, primitive families of urodelans such as Protidae, in which a blood cell forming bone marrow has still not appeared, contain lympho-haemopoietic tissue in both pro- and meso-nephros throughout all their lives. In contrast, in the most evolved family, Plethodontidae, in which haemo-

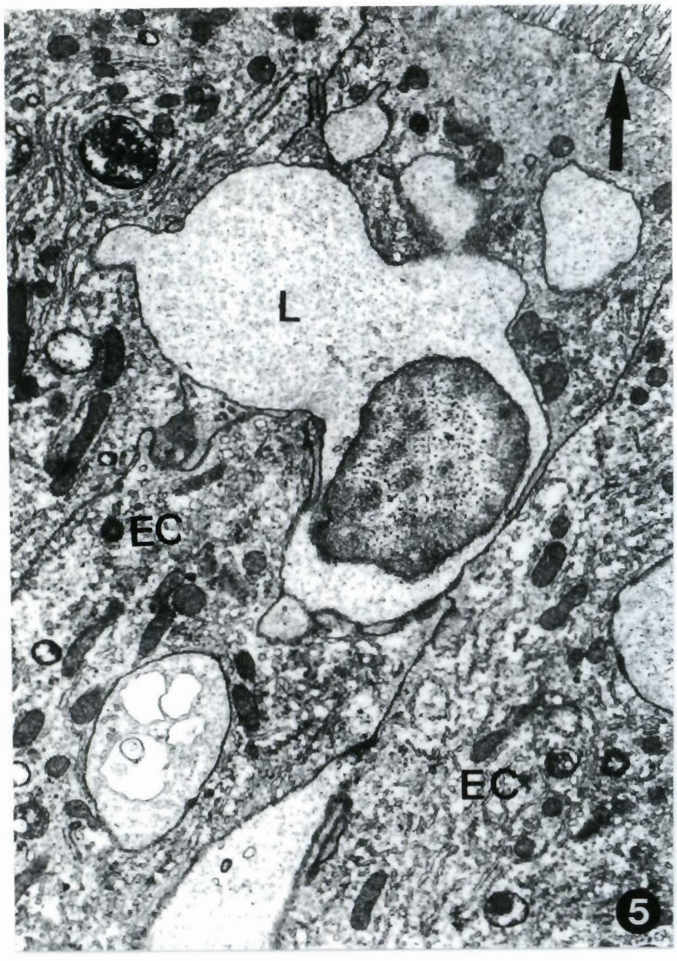


Fig. 5. Electron-lucent intraepithelial lymphocyte (L) in the gut of Atlantic hagfish, *Myxine glutinosa*. Epithelial cells (EC), microvilli (arrow). x 7,500

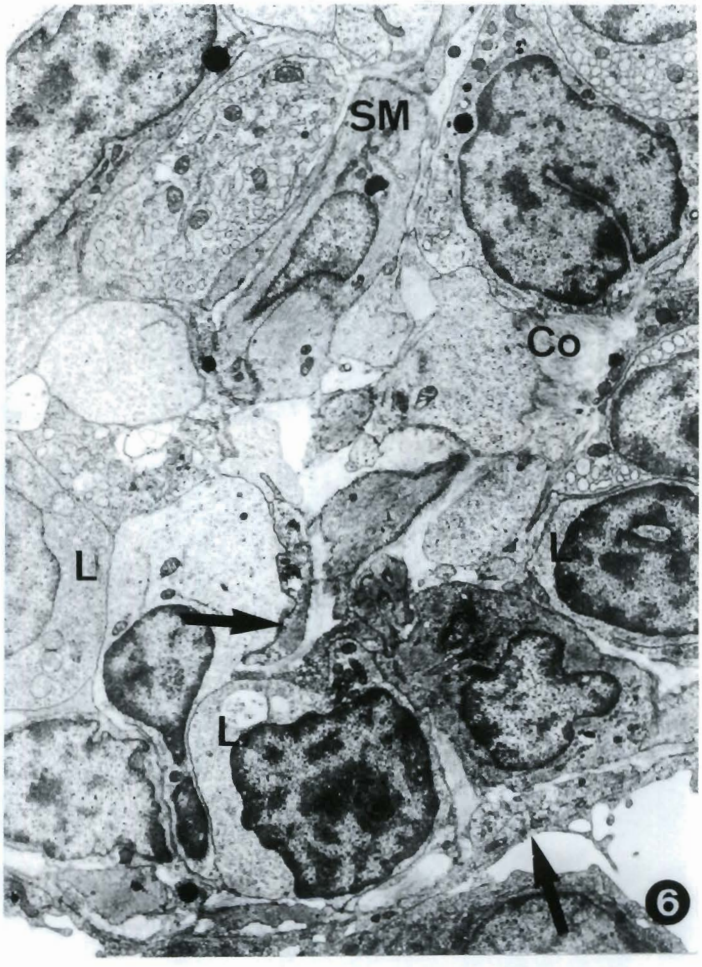


Fig. 6. Group of lymphoid cells (L) in the gut lamina propria of the skate, *Raja clavata*. Smooth muscle cell (SM), collagen fibres (Co), fibroblastic cell processes (arrows). x 3,750

poietic bone marrow is present, the kidney is not capable of producing blood cells (Curtis et al., 1979; Manning and Horton, 1982). Moreover, in adult anurans with bone marrow, the kidney, which had formed blood cells during both embryonic and larval life, loses its lympho-haemopoietic activity (Du Pasquier, 1968; Horton, 1971), although some primitive families, such as Pipidae, maintain the primitive perihepatic cell layer active in granulopoiesis (Hadji-Azimi and Fischberg, 1967; Manning and Horton, 1969; Hadji-Azimi et al., 1982).

In most adult elasmobranches, the kidney is not a lympho-haemopoieitic organ (Fange, 1982, 1984; Zapata and Cooper, 1990), but during ontogeny the nephrogenic mesenchyme close to the postcardinal vein is one of the first intraembryonic loci to contain primitive blood cells. We and other authors (Lloyd-Evans, 1993) have analyzed these changes in the lympho-haemopoietic activity of elasmobranch kidney during ontogeny of the dogfish, *Scyliorhynus canicula* and have noticed the importance of cell microenvironment in determining the functionality of these primitive lympho-haemopoietic

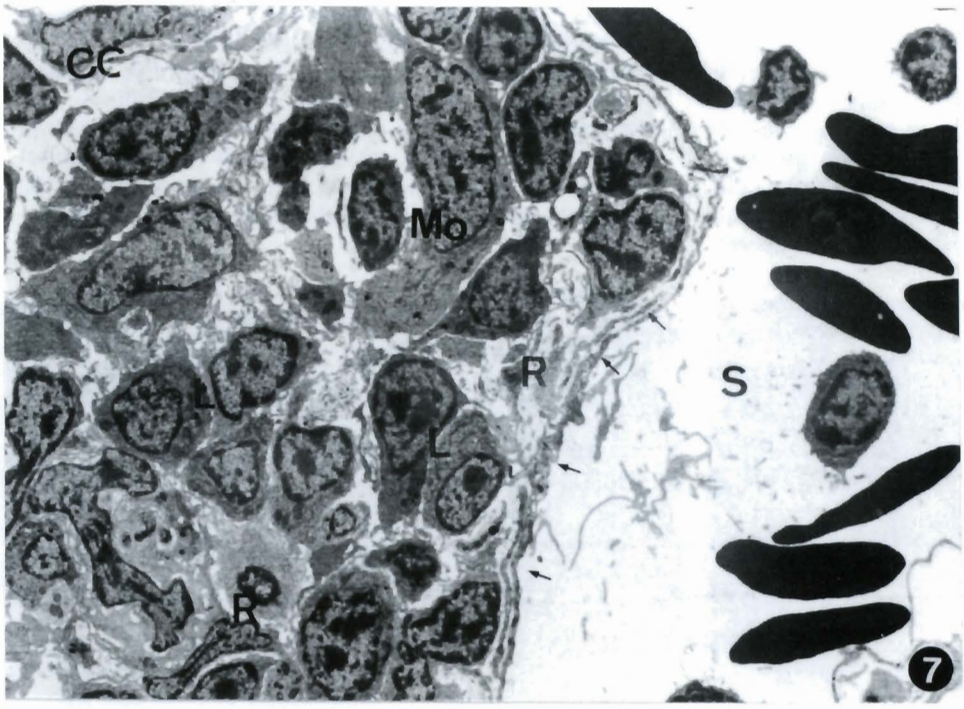


Fig. 7. Jugular body of *Rana perezi*. Cell cords (CC), consisting largely of lymphocytes (L) and monocytes (Mo) in a network of reticular cell processes (R), appear arranged between sinusoidal blood vessels (S). Endothelial cells (arrows). x 2,400

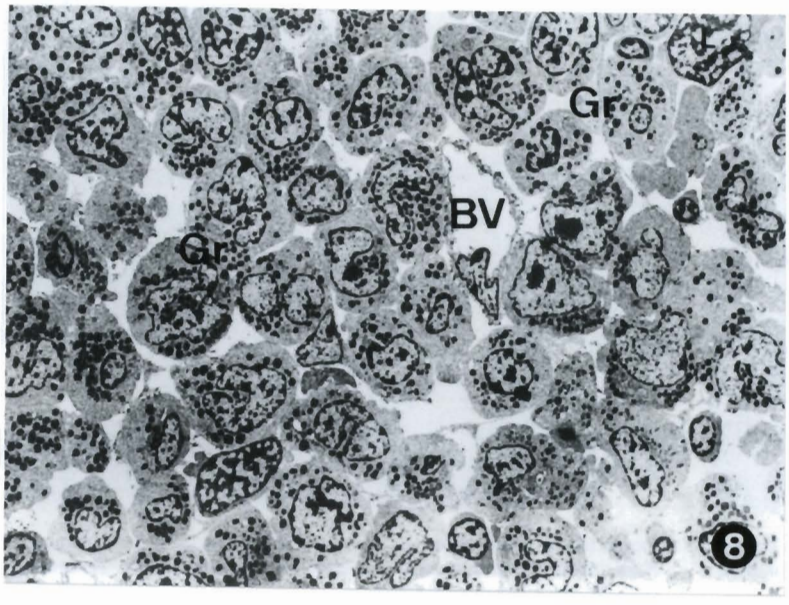


Fig. 8. Masses of lympho-haemopoietic tissue containing mainly mature and developing granulocytes (Gr) in the Leydig organ of an embryonic dogfish, *Scyliorhinus canicula*. Blood vessel (BV). x 1,100

organs.

The first lympho-haemopoietic cell progenitors in embryonic dogfish appear in the walls of the extra embryonic yolk sac in animals 1 cm long belonging to the so-called stage I (Figs. 16a,b). A short time later, in the same developmental stage (embryos of 1.5-2 cm), a thymic epithelial primordium can be identified and Ig-positive cells are demonstrated, using an anti-IgM antiserum, in the embryonic liver (Lloyd-Evans, 1993). Early in stage II (2-2.5 cm long embryos) groups of haemocytoblasts can be found, by both light and electron microscopy, in the nephrogenic mesenchyme which surrounds the postcardinal vein (Fig. 17). At this moment the first lymphoid elements colonize the thymic primordium but there appears to be no haemopoietic activity in other organs, including the Leydig and epigonal organs. The kidney is therefore the first peripheral tissue to become lympho-haemopoietic in dogfish embryo, a fact also reported by other authors (Hart et al., 1988; Lloyd-Evans, 1993). In embryos 2.5-3.5 cm in length, mature and developing granulocytes occupy the renal parenchyma, and lymphoid cells rapidly appear in the organ. However, in stage III embryos (5-10 cm in length), the renal lympho-haemopoietic activity starts to decline (Fig. 18), whereas other peripheral lymphoid organs, mainly Leydig and epigonal organs, reach the adult condition. Finally, in the last stages of embryonic life, there is a total lack of lympho-haemopoietic activity in the dogfish kidney (Fig. 19).

The most primitive vertebrates, Agnatha, have no true lymphoid organs

The condition in Agnatha is, as mentioned previously, striking. The cyclostomes seem able to respond, albeit poorly, to both T-dependent and T-independent antigens, although the molecules involved in the responses, especially in the case of the hagfish have yet to be confirmed (Du Pasquier, 1993a). In these primitive animals neither MHC molecules (Kronenberg et al., 1994) nor immunoglobulins have been found (Litman et al., 1992; Du Pasquier, 1993a) and, on the contrary, in the Pacific hagfish, a member of the C3-like complement protein family has been claimed to be

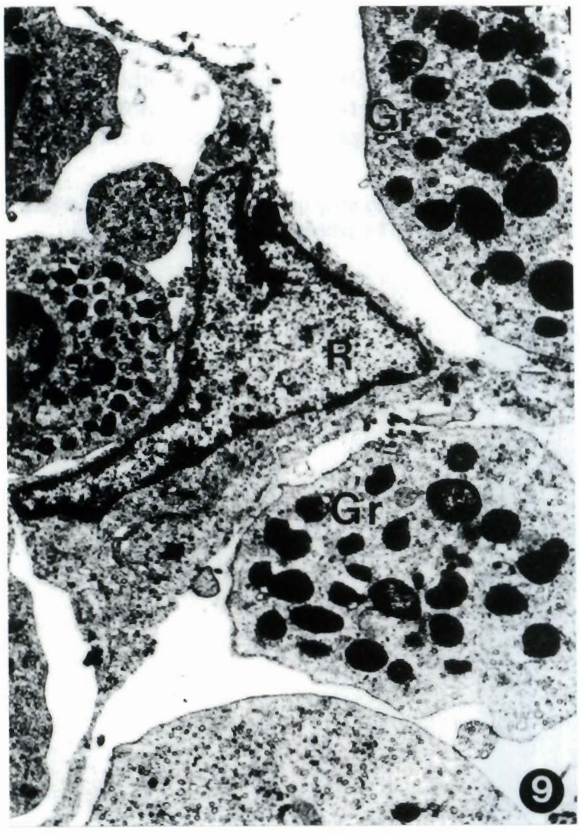


Fig. 9. Reticular cell (R) which organizes the supporting network of the bone marrow-equivalent lympho-haemopoietic organs of lower vertebrates, in a cell cord of dogfish Leydig organ. Granulocytes (Gr). x 5,200

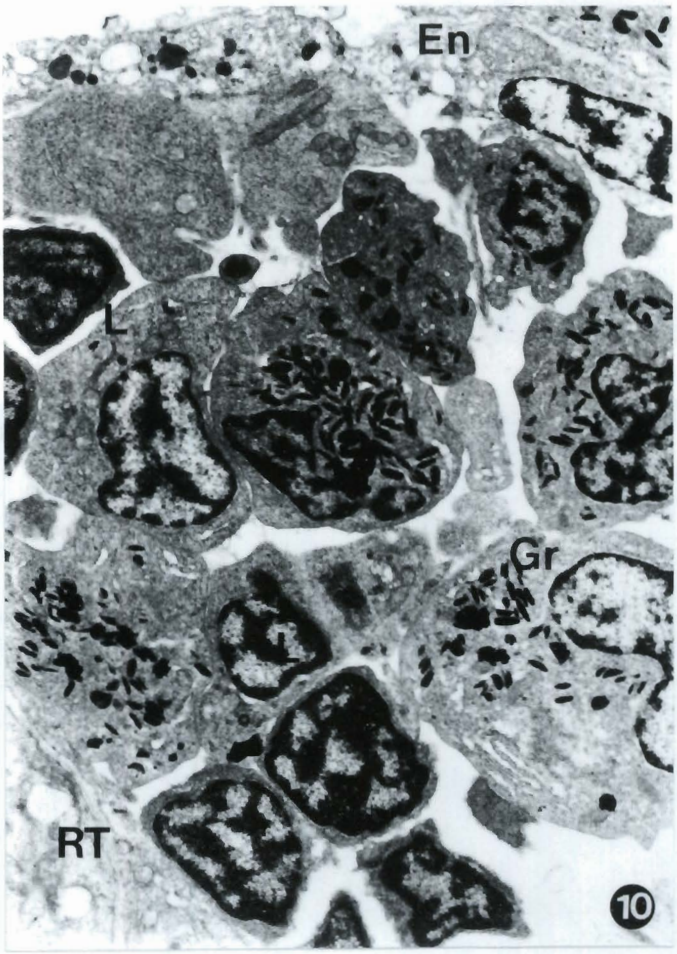
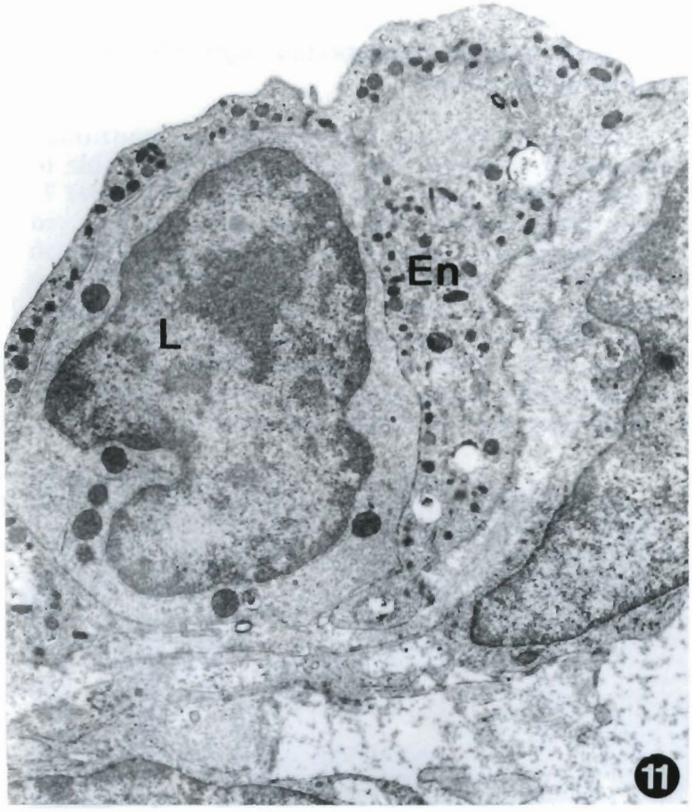


Fig. 10. Group of granulocytes (Gr) and lymphoid cells (L) in a cell cord arranged between the renal tubules (RT) and the endothelium (En) of a sinusoidal blood vessel. Mesonephros of *Gobio gobio*. x 3,500



involved in non-anticipatory responses, more related to the non-specific defence mechanisms of invertebrates than to the sophisticated immune responses of vertebrates (Hanley et al., 1992; Ishiguro et al., 1992; Raison et al., 1994). However, cells morphologically similar to lymphocytes circulate in the hagfish blood and we described ultrastructurally clusters of lympho-haemopoietic tissue, including plasma cells, in the pronephros (Zapata et al., 1984), an organ classically claimed to be the equivalent of the thymus of Gnathostomata, of the Atlantic hagfish, *Myxine glutinosa* (Fange, 1966). Other authors have failed however, to identify plasma cells in various species of Pacific hagfish, including *Eptatreturs burgeri* (Tomonaga and Fujii, 1994). In the light of these recent data on the lack of immunoglobulins in Pacific hagfish, the finding of plasma cells in the Atlantic species raises a major controversy and thus requires further confirmation (Du Pasquier, 1993a). Although lampreys seem to contain typical Ig H and L chains, the nature of these L chains must also be confirmed. Furthermore, the two H and L subunits are not covalently joined together as in other Ig molecules, and lamprey Ig genes are still to be characterized (Litman et al., 1992). However, various research groups have recorded the occurrence of plasma cells in this species (Kilarski and Plytycz, 1981; Zapata et al., 1981a; Fujii, 1982; Hagen et al., 1983) and the opsonizing effect of their Ig molecule has also been demonstrated (Fujii, 1981). Therefore, although hagfish and lampreys belong to the same taxonomic class of vertebrates, they are representative of two very different

Fig. 11. Lymphocyte (L) migrating through the endothelial wall (En) of a sinusoidal blood vessel in the pronephros of a teleost. x 7,500

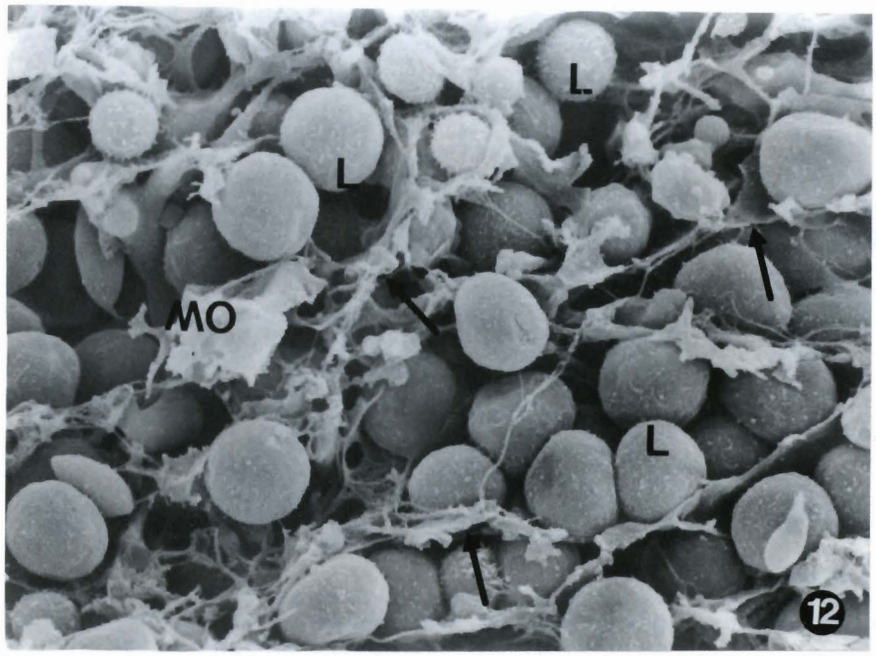
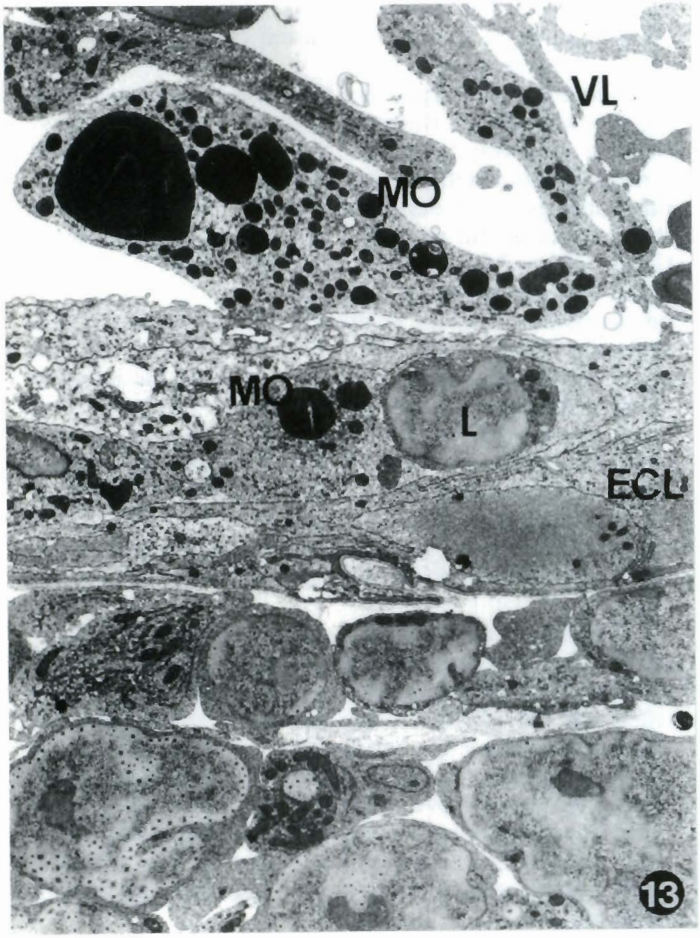


Fig. 12. Different-sized lymphoid cells (L) and macrophages (MO) arranged among the cell processes of reticular cells (arrows) in the meningeal lympho-haemopoietic tissue of stingray, *Dasyatis akajei*. x 2,250



orders of cyclostomes which seem to be considerably different from an immunological point of view.

On the other hand, neither hagfish nor lampreys possess a morphologically identifiable thymus and secondary lymph organs consist of lympho-haemopoietic tissues morpho-functionally equivalent to the bone marrow. In the Atlantic hagfish, the pronephros, a primitive organ which filters the content of the pericardial cavity via nephrostomes in the so-called central mass (Holmgren, 1950; Fange and Zapata, 1985), was considered to be the equivalent of the thymus (Fange, 1966). However, ultrastructural analysis of the pronephros of several specimens of different sizes of Atlantic hagfish, *Myxine glutinosa*, demonstrated that the central mass is really an epithelial filtering organ with some areas of active erythropoiesis in which macrophages and typical plasma cells are found (Fig. 20) (Zapata et al., 1984; Fange and Zapata, 1985). Other authors described lymphocyte-like cells in the muscle-velum complex of Pacific hagfish as well as thymus equivalent (Riviere et al., 1975). We think however, that these are more similar to the satellite cells of the skeletal muscle. So, although the issue of the hagfish thymus remains unclear because early developmental stages of these animals are not accessible, we believe, in agreement with classical reports (Papermaster et al., 1964; Kampmeier, 1969) that they have no true thymus. On the other hand, lympho-haemopoietic tissue masses

Fig. 13. Macrophage-lymphocyte cell clusters found in the hypothalamic ventricle of some specimens of elasmobranches. Macrophages (MO) and lymphocytes (L) cross the ependyme cell layer (ECL) colonizing the ventricular lumen (VL). x 4,500

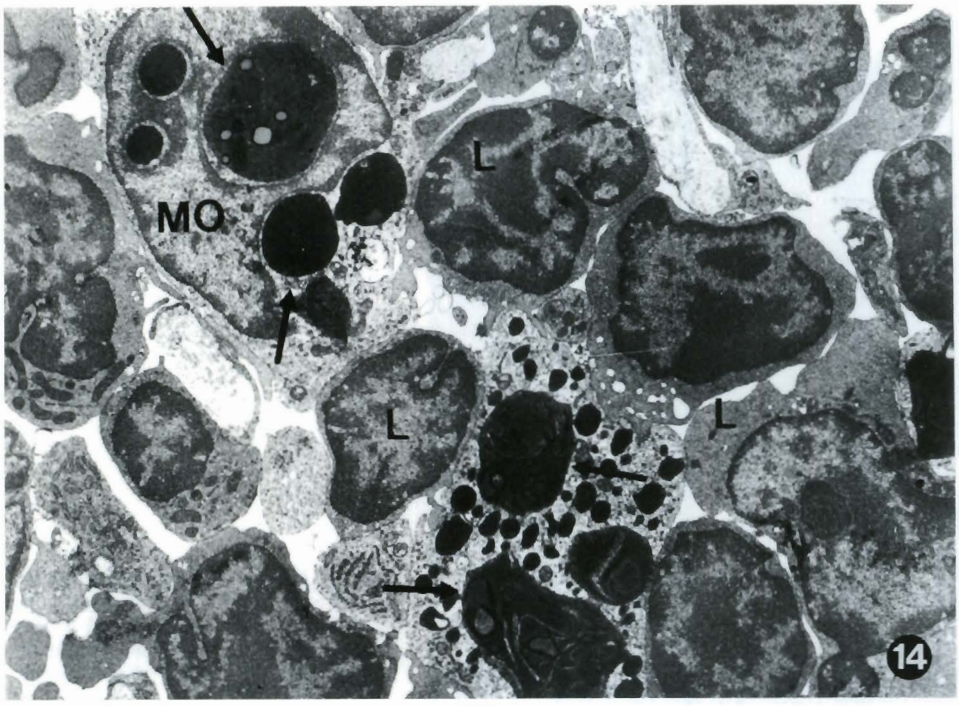


Fig. 14. Macrophage-lymphocyte cell cluster in the ventrical lumen of the hypothalamus of *Triakis scyllia*. Note the abundant cell debris (arrows) engulfed by macrophages (MO) and the close contacts with the neighbouring lymphocytes (L). x 4,500

occupy the intestinal submucosa of hagfish (Tomonaga et al., 1973; Tanaka et al., 1981; Fange and Zapata, 1985). These consist largely of developing and mature granulocytes arranged between large fat cells in a supporting network of fibroblastic reticular cells (Fig. 21).

In lampreys, lymphoid accumulations identified by light microscopy in the branchial region were presented as possibly homologous of the thymus (Finstad et al., 1984). After careful examination of their ultrastructure, we and other authors demonstrated that they actually represent filtering regions in which material present in the pharyngeal lumen is actively captured by the phagocytic blood vessel endothelium and/or circulating macrophages (Fig. 22) (Page and Rowley, 1982; Ardavín and Zapata, 1988). Therefore, in absence of the thymus, the main lymphoid organs of lampreys are lympho-haemopoietic tissues which occur in different organs throughout the complex life cycle of these animals, reemphasizing the importance of haemopoietic inductive cell microenvironments in the functioning of lympho-haemopoietic organs of primitive vertebrates (Percy Lord and Potter, 1977; Tanaka et al., 1981; Ardavín et al., 1984; Ardavín and Zapata, 1987).

In ammocoetes, the larval form of lampreys, the typhlosole, a fold of the mid gut described by some authors as a primitive spleen (Tanaka et al., 1981), the nephric fold plus the larval opistonephros and the neighbouring adipose tissue represent the main lympho-haemopoietic organs (Fig. 23). All of these have the same histological organization consisting of cell cords which home all the blood cell lineages, including lymphocytes and plasma cells, arranged between sinusoidal blood vessels and, in the case of the kidney, renal tubules (Fig. 24).

During metamorphosis, these organs, especially the typhlosole which disappears completely, regress, because the loose connective tissue which supported the lympho-haemopoietic tissue is substituted by fibroblasts, macrophages and dense masses of collagen fibres, thus reducing the presence of lympho-haemopoietic tissue (Fig. 25). After metamorphosis, when the adult opistonephros is organized, the lympho-haemopoietic activity re-appears there. At this stage, and previously, just after metamorphosis, the so-called supraneural body, a fat column along the lamprey central nervous system which has no haemopoietic activity in ammocoetes, begins to home progenitor cells, becoming the most important blood cell-forming organ of adult lampreys. The histological organization of the supraneural body of adult lampreys is very similar to that of mammalian bone marrow with cell cords arranged among enormous fat cells (Fig. 26).

Therefore, changes in the cell microenvironments of different organs determine their capacities for housing lympho-haemopoiesis throughout the lamprey life cycle (see Table 1). Presumably, a similar process occurred during the evolution of vertebrates. In those vertebrates which colonized the land, thus losing the protective properties of water, the bone with its radioprotective capacities provided the safest cell microenvironment for homing the haemopoietic stem cells, known to be extremely sensitive to radiation.

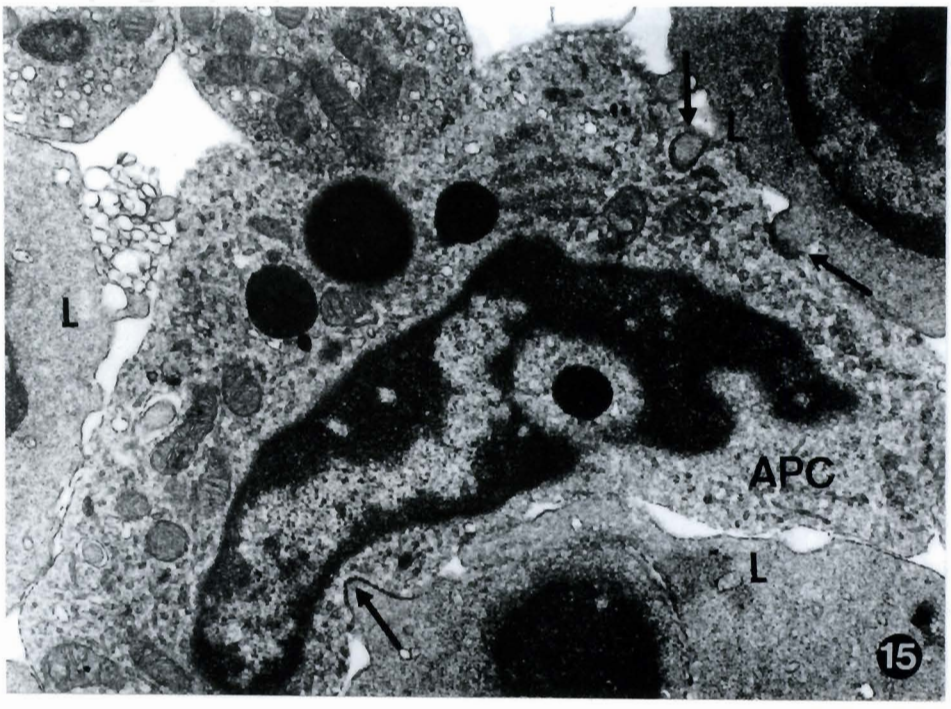


Fig. 15. Antigen-presenting cells (APC) establish close contacts (arrows) with the neighbouring lymphocytes (L) in the macrophage-lymphocyte cell clusters of the hypothalamus of *Scyliorhinus torazame*. x 12,000

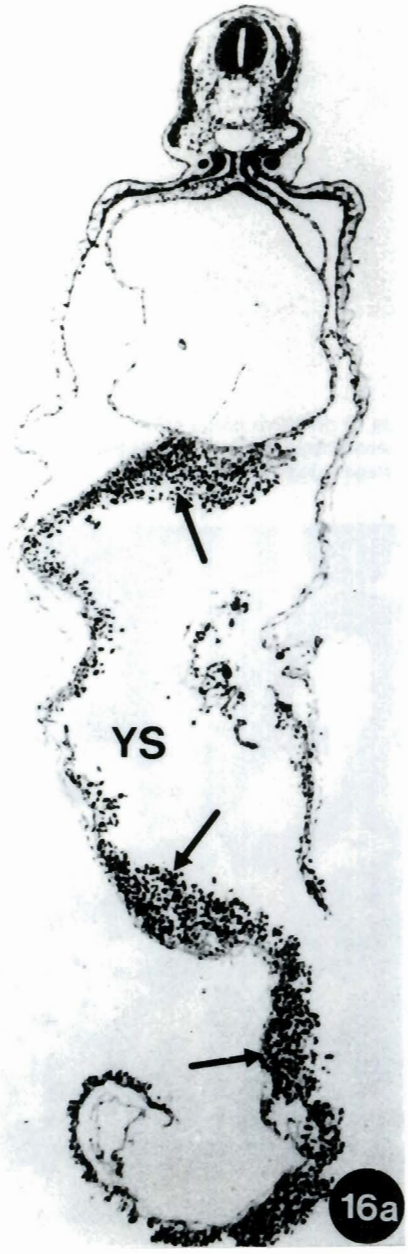
Table 1. Changes in the lympho-hemopoietic loci throughout the life span of the lamprey *Petromyzon marinus*.

	AMMOCOETES			PREMETAMORPHOSING AMMOCOETES	MACROPHTHALMIA STAGE	PARASITIC ADULT
	Stage I	Stage II	Stage III	Stage IV	Stage V	Stage VI
Typhlosole	+++	+++	++	+	-	-
Nephric fold plus larval opisthonephros	++	+++	+	+/-	-	-
Adipose tissue	+++	++	-	-	-	++
Adult opisthonephros	-	-	-	-	-	++
Supraneural body	-	-	-	+	++	+++

The lack of germinal centres in the immune system of ectotherms newly reflects the importance of cell microenvironments for the evolution of the vertebrate immune system

Like the occurrence of numerous lympho-haemopoietic tissues equivalent to bone marrow, another striking feature of the lymphoid organs of lower vertebrates, the lack of germinal centres, again demonstrates the importance of cell microenvironment for determining the functional capacities of different organs; in this case, their immunological capabilities (Zapata and Cooper, 1990; Nahm et al., 1992; Du Pasquier, 1993a).

In higher vertebrates, germinal centres histologically define the cell microenvironment in which stimulated B lymphocytes are selected after undergoing somatic hypermutation for improving the affinity of their antigen



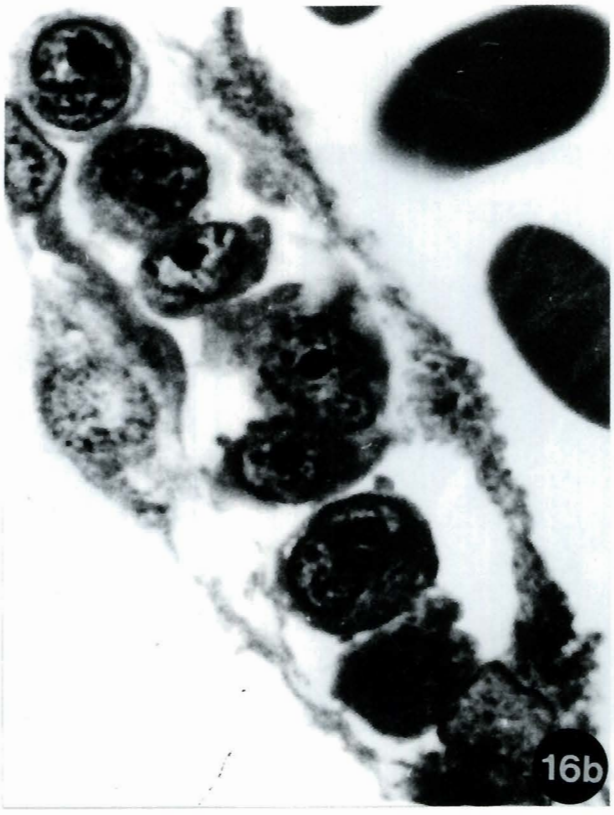


Fig. 16. a. Section of an early dogfish embryo showing the yolk sac (YS) in the walls of which (arrows) appear numerous primitive blood cells. x 20. **b.** Detail of these primitive cells. x 1,900

receptors (Nossal, 1994). Furthermore, some of the selected B cells show isotypic switching and are accumulated as memory cells. On the contrary, in all lower vertebrates the Ig repertoire is limited and antibody affinity does not increase with immune response (Du Pasquier, 1993b). In addition, the immunological memory is poor and isotype switching only seems possible in some species (Du Pasquier, 1993b). For many years, authors considered that these limitations stemmed from the lack of a high number of V segments and/or the absence of somatic mutations (Du Pasquier, 1982). Recent data, however, have demonstrated that, apart from Agnatha, all lower vertebrates including Chondrichthyes, are capable of considerable somatic mutation (Du Pasquier, 1993b). The poor ectotherm immune response is thus due to the lack of an efficient mechanism for selecting the mutants resulting from the absence of germinal centres (Du Pasquier, 1993b).

It is therefore pertinent to ask why there are no

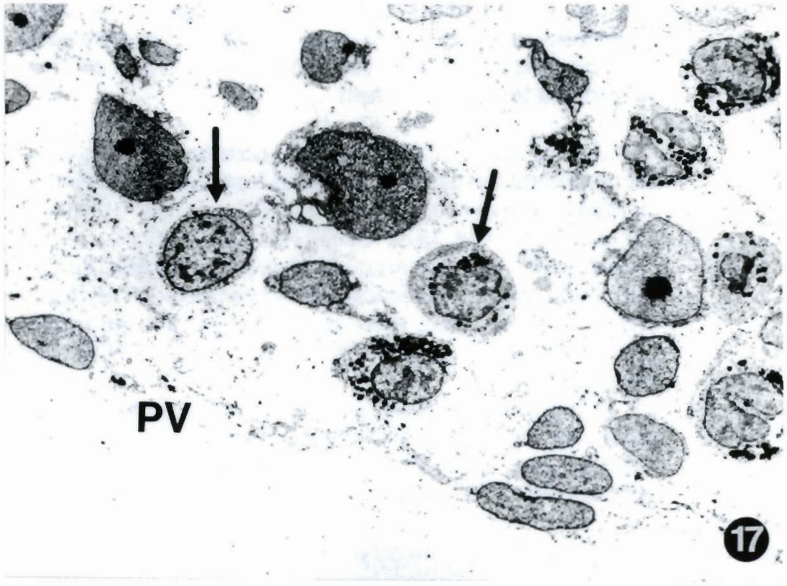


Fig. 17. Group of primitive blood cells (arrows) in the nephrogenic mesenchyme which surrounds the postcardinal vein (PV) of a stage II dogfish embryo. x 1,100

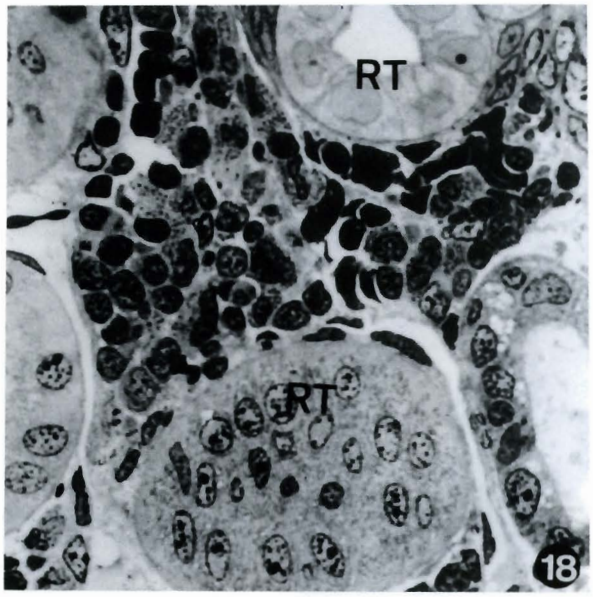


Fig. 18. Small groups of lympho-haemopoietic cells occur among the renal tubules (RT) of a stage III dogfish embryo. x 800

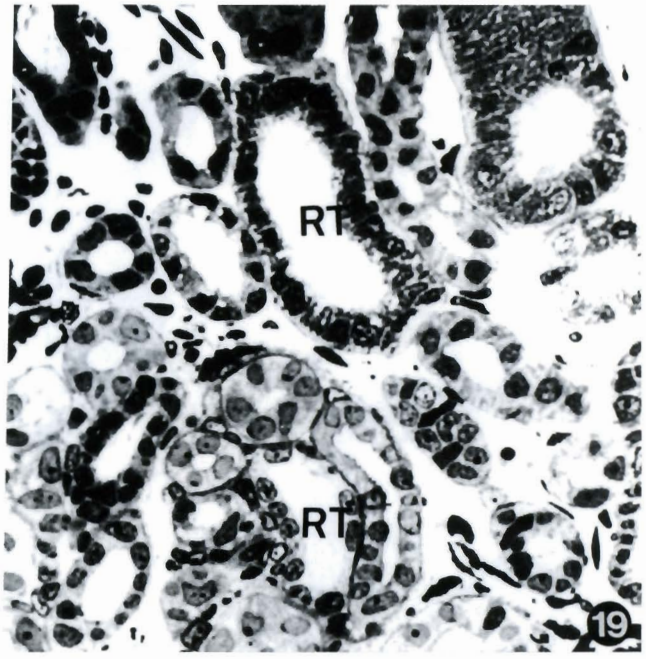


Fig. 19. Absence of lympho-haemopoietic tissue in the adult dogfish kidney. Renal tubules (RT). x 600

germinal centres in lower vertebrates. Some authors suggested that melanomacrophage centres occurring in most teleosts are their phylogenetic precursors (Lamers, 1985). According to our own results, melanomacrophage centres merely represent scavengers in the teleost lymphoid organs in which macrophages filled with degraded materials, including metabolic debris and even antigens, accumulate (Herraez and Zapata, 1986). They are neither morphologically nor functionally related to the germinal centres of higher vertebrates. We can therefore suggest that the absence of germinal centres in lower vertebrates is related to the lack of all or some of the elements necessary for organizing germinal centres in mammals. As already known, B cells, follicular

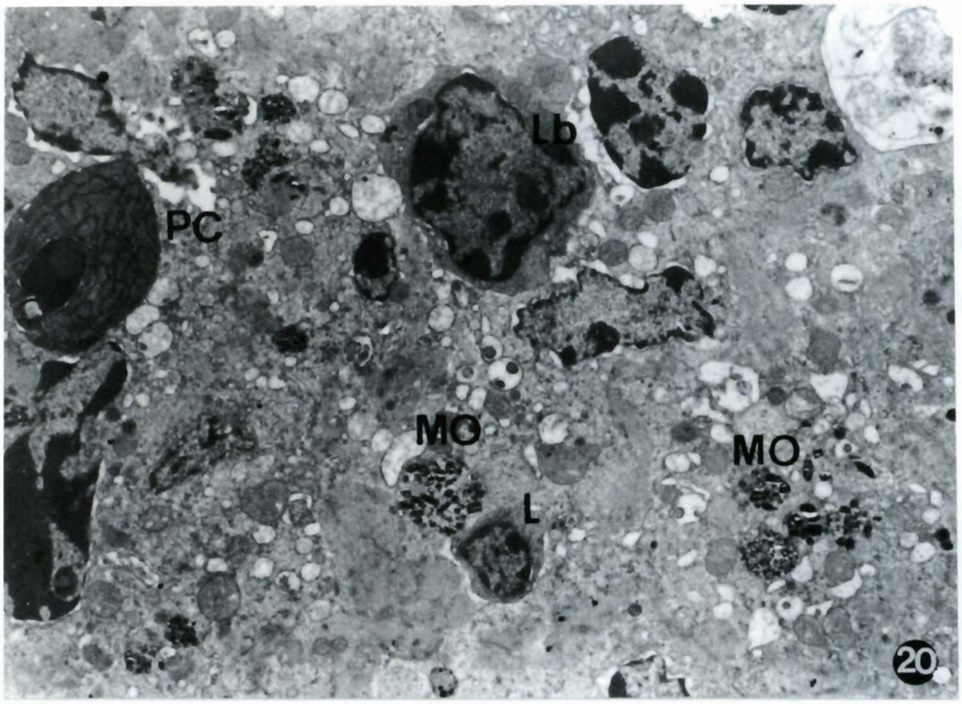


Fig. 20. Lympho-haemopoietic tissue in the pronephric central mass of an Atlantic hagfish, *Myxine glutinosa*. Macrophages (MO), lymphocytes (L), lymphoblasts (Lb), degenerated plasma cells (PC). x 3,500

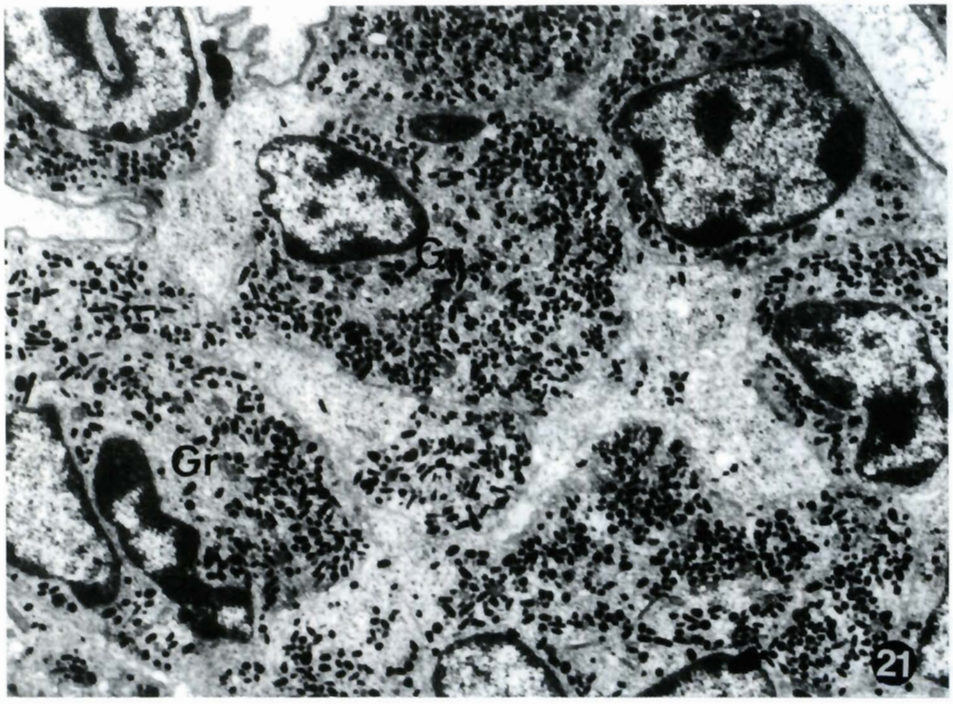


Fig. 21. Masses of mature and developing granulocytes (Gr) in the intestinal lamina propria of *Myxine glutinosa*. x 4,500

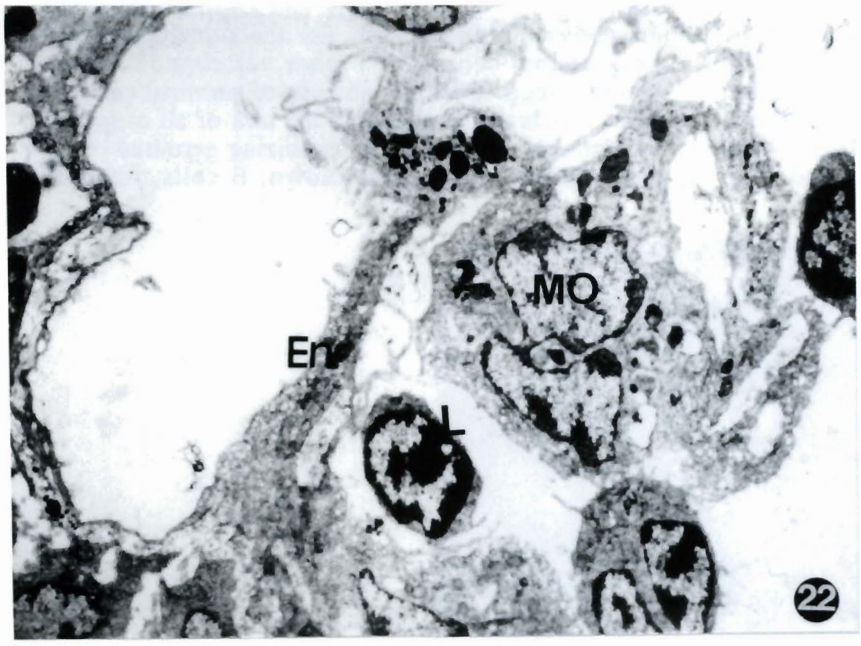


Fig. 22. Lymphoid tissue of the branchial region of the anadromous sea lamprey, *Petromyzon marinus* actually represents filtering areas consisting of large macrophages (MO), lymphocytes (L) and phagocytic endothelia (En). x 3,500

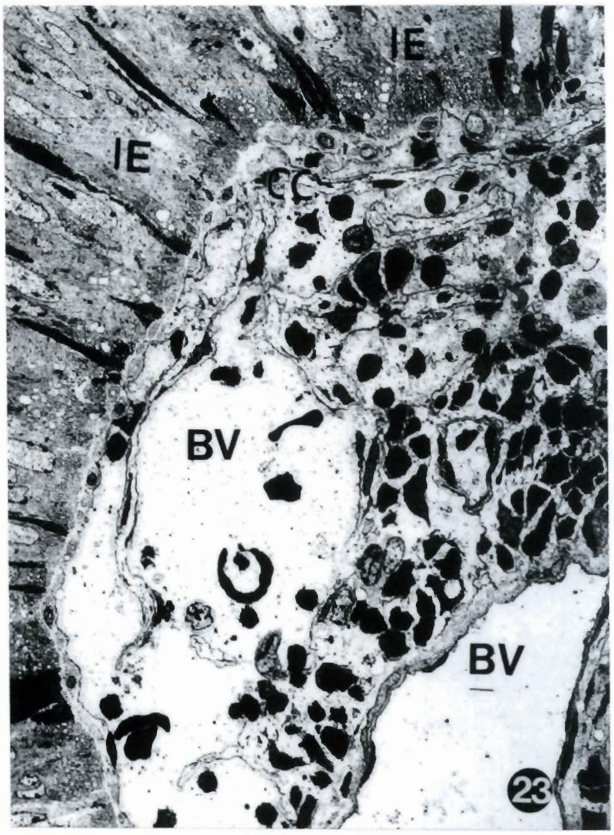


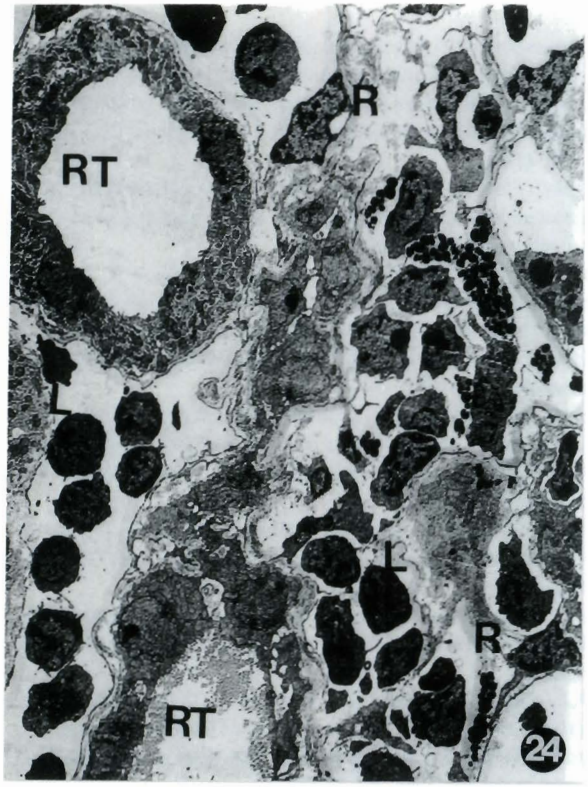
Fig. 23. Cell cords (CC) which contain mature and developing blood cells, including lymphocytes and plasma cells, and enlarged sinusoidal blood vessels (BV) in the thyphlosolar lamina propria of an ammocoete of *Petromyzon marinus*. Intestinal epithelium (IE). x 800

dendritic cells, capable of retaining and presenting immune complexes, and a few T cells involved in the production of specific cytokines are the main cell components of mammalian germinal centres. We know very little about the T cell subsets of lower vertebrates, but cells capable of retaining immune complexes have been described in several non-mammalian species (Diener and Nossal, 1966; Ellis, 1980; Baldwin and Cohen, 1981; Kroese and van Rooijen, 1983; Kroese et al., 1985; Leceta and Zapata, 1991). On the other hand, B lymphocytes occurring in lower vertebrates could be equivalent to the B-1 cells of mammals, which express the T cell marker CD5, show a very restricted repertoire and are unable to generate germinal centres (Nahm et al., 1992). Unfortunately, we have very little information on the phylogenetic origins of B cell subsets. Recently, Horton et al. (1994) reported, by using a monoclonal antibody specific to *Xenopus* CD5, that early thymectomy in *Xenopus* tadpoles eliminated all the splenic CD5-positive cells. These results need, however, further confirmation, because B1 CD5-positive cells are also very scarce in the mammalian spleen, accumulating principally in the peritoneal cavity.

Concluding remarks

We can conclude that both the appearance and evolution of distinct lymphoid organs through vertebrate evolution which are intimately associated with the emergence of immune capacities, are strongly influenced by the non-lymphoid cell components of their inductive cell microenvironments.

Thymus, spleen and gut-associated lymphoid aggregates appear early in phylogeny. Chondrichthyes,



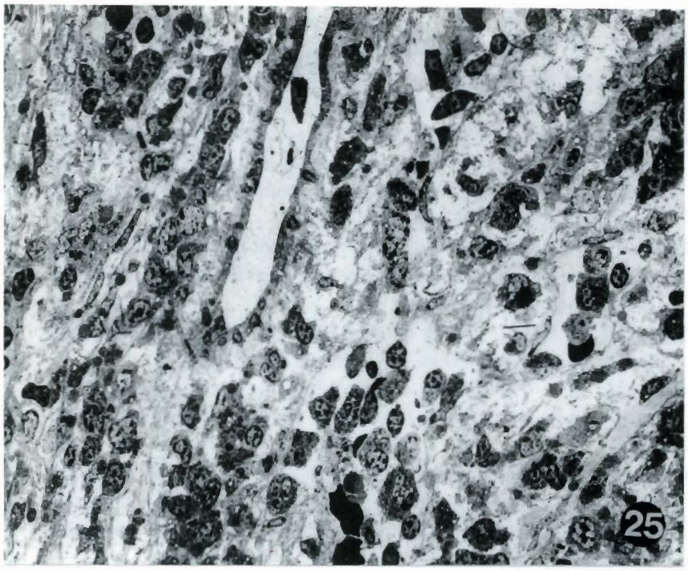


Fig. 25. Metamorphosing thyphlosole of *Petromyzon marinus*. Increasing amount of connective tissue reduces the presence of lympho-haemopoietic tissue. x 800

Fig. 24. Lympho-haemopoietic tissue among the renal tubules (RT) of larval opistonephros of *Petromyzon marinus*. Lymphoid cells (L), fibroblastic reticular cells (R). x 1,500

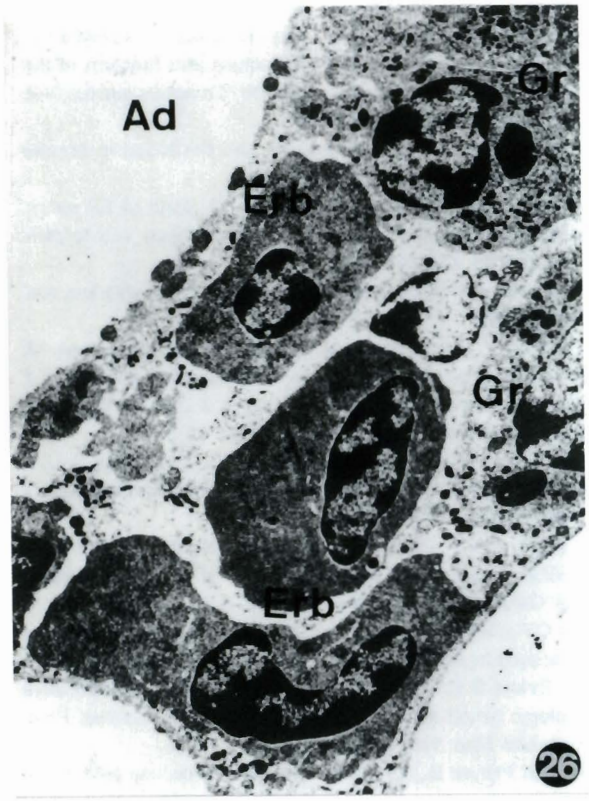


Fig. 26. Granulocytes (Gr) and erythroblasts (Erb) among big fat cells (Ad) in the supraneural body of an adult lamprey, *Petromyzon marinus*. x 3,000

which contain MHC molecules and a primitive organization of Ig genes, are the first vertebrates to show such lymphoid organs. Agnatha, mainly hagfish, the immune capacities of which need profound revision, have no true lymphoid organs.

The bone marrow as well as the lymph nodes are late phylogenetical adquisitions of the vertebrate immune system. The former appears in the most evolved urodeles of the Plethodontidae family, whereas true lymph nodes occur only in some birds and mammals. However, primitive vertebrates mimic the bone marrow haemopoietic cell microenvironments to support lympho-haemopoiesis in numerous unrelated loci such as gonads, gut, kidney, brain, etc ...

Finally, the lack of germinal centres in ectothermic vertebrates impedes the selection of B cell mutants raised after somatic hypermutation, resulting in a poor immunological memory and low increase of antibody affinity throughout the immune response. Further research to improve the knowledge of cell components of peripheral lymphoid organs in lower vertebrates could clarify the origin of this remarkable absence.

References

Ardavín C.F. and Zapata A.G. (1987). Ultrastructure and changes during metamorphosis of the lympho-hemopoietic tissue of the larval anadromous sea lamprey *Petromyzon marinus*. Dev. Comp. Immunol. 11, 79-93.

Ardavín C.F. and Zapata A.G. (1988). The pharyngeal lymphoid aggregates of lampreys: a morpho-functional equivalents of the vertebrate thymus? Thymus 11, 59-65.

Ardavín C.F., Gomariz R.P., Barrutia M.G., Fonfría J. and Zapata A.G. (1984). The lymphohemopoietic organs of the anadromous sea lamprey *Petromyzon marinus*. A comparative study throughout its life span. Acta Zool. Stockh. 65, 1-15.

Baldwin III W.M. and Cohen N. (1981). A giant cell with dendritic cell properties in spleen of the anuran amphibian *Xenopus laevis*. Dev. Comp. Immunol. 5, 416-474.

Bjerring H.H. (1984). The term «Fosse Bridjei» and five endocranial fossae in Telestome fishes. Zool. Scripta 13, 231-238.

Chandler A.C. (1911). On a lymphoid structure lying over the myelencephalon of Lepisosteus. Univ. California Publ. Zool. 9, 85-104.

Chibá A., Torroba M., Honma Y. and Zapata A.G. (1988). Occurrence of lympho-haemopoietic tissue in the meninges of the stringray *Dasyatis akajei* (Elasmobranchii, Chondrichthyes). Am. J. Anat. 183, 268-276.

Cooper E.L. (1976a). Comparative immunology. Prentice Hall. Englewood clifts. New Jersey.

Cooper E.L. (1976b). Immunity mechanisms. In: Physiology of the amphibia. Vol. 3. Lofts B. (ed). Academic Press. New York. pp 163-272.

Curtis S.K., Cowden R.R. and Knagel J.W. (1979). Ultrastructure of the bone marrow of the salamander *Plethodon glutinosus* (Caudata Plethodontidae). J. Morphol. 123, 405-440.

Dempster W.T. (1930). The morphology of the amphibian endolymphatic organ. J. Morphol. 50, 71-126.

Diener E. and Nossal G.J.V. (1966). Phylogenetic studies on the immune responses. Localization of antigens and immune response in toad *Bufo marinus*. Immunology 19, 535-542.

Du Pasquier L. (1968). Les proteins sèriques et le complex lymphomyeloïde chez le tetard d'*Alytes obstatricaus* normal et thymectomisé. Anst. Inst. Pasteur. 114, 490-502.

Du Pasquier L. (1982). Antibody diversity in lower vertebrates why is it so restricted? Nature 296, 311-316.

Du Pasquier L. (1993a). Evolution of the immune system. In: Fundamental immunology. Third edition. Paul W.E. (ed). Raven Press, Ltd. New York. pp 199-233.

Du Pasquier L. (1993b). Phylogeny of B-cell development. Curr. Opin. Immunol. 5, 185-193.

Ellis A.E. (1980). Antigen-trapping in the spleen and kidney of the plaice, *Pleuronectes platessa*. J. Fish Dis. 3, 413-426.

Fange R. (1966). Comparative aspects of excretory and lymphoid tissue. In: Phylogeny of immunity. Smith R.T., Miescher P.A. and Good R.A. (eds). University of Florida Press. Gainesville. pp 140-145.

Fange R. (1982). A comparative study of lymphomyeloid tissue in fish. Dev. Comp. Immunol. Suppl. 2, 23-33.

Fange R. (1984). Lymphomyeloid tissues in fishes. Vidensk. Meddr. Dansk. Naturh. Foren. 145, 143-162.

Fange R. and Zapata A.G. (1985). Lymphohemopoietic system and bodily defences of hagfish. In: Ontogeny and phylogeny of immune system. The Iwanami Immunology Series. Vol. 4. Good R.A. (ed). Iwanami Shoten Publ. Japan. pp 59-76.

Fichtelius K.E., Finstad J. and Good R.A. (1968). Bursa equivalents of bursaless vertebrates. Lab. Invest. 19, 339-351.

Finstad J., Papermaster B. and Good R.A. (1964). Evolution of the immune responses. II. Morphologic study on the origin of the thymus and organized lymphoid tissue. Lab. Invest. 13, 490-512.

Fujii T. (1981). Antibody-enhanced phagocytosis of lamprey polymorphonuclear leucocytes against sheep erythrocytes. Cell Tissue Res. 219, 41-51.

Fujii T. (1982). Electron microscopy of the leucocytes of the typholosole in ammocoetes with special attention to the antibody producing cells. J. Morphol. 173, 87-100.

Good R.A., Finstad J., Pollara B. and Gabrielsen A.E. (1966). Morphological studies of the evolution of the lymphoid tissues among the lower vertebrates. In: Phylogeny of immunity. Smith R.T., Miescher P.A. and Good R.A. (eds). University of Florida Press. Guinesville. pp 149-167.

Hadji-Azimi I. and Fischberg M. (1967). Hematopoiese perihepatique chez le batracien anoure *Xenopus laevis*. Comparaison entre les individues normaux et les porteurs de tumeurs lymphoïdes. Rev. Suisse Zool. 74, 642-647.

Hadji-Azimi I., Schwager J., Thiebard C.H. and Perrenot N. (1982). B-lymphocyte differentiation in *Xenopus laevis larvae*. Dev. Biol. 90, 253-258.

Hagen M., Filosa M.F. and Youson J.H. (1983). Immunocytochemical localization of antibody-producing cells in adult lamprey. Immunol. Lett. 6, 87-92.

Hanley P.J., Hook J.W., Raftos D.A., Gooley A.A., Trent R. and Raison R.L. (1992). Hagfish humoral defense protein exhibits structural and functional homology with mammalian complement components. Proc. Natl. Acad. Sci. USA. 89, 7910-7914.

Hart S., Wrathmell A.B., Harris J.E. and Grayson T.H. (1988). Gut immunology in fish: a review. Dev. Comp. Immunol. 12, 453-480.

Herraez M.P. and Zapata A. (1986). Structure and function of the melano-macrophage centres of the goldfish, *Carassius auratus*. Vet. Immunol. Immunopathol. 12, 117-126.

Holmgren N. (1950). On the pronephros and the blood in *Myxine glutinosa*. Acta Zool. Stockh. 31, 233-248.

Horst C.J. van der (1925). The myelencephalic gland of *Polyodon, Acipenser* and *Amia*. Konink. Akad. Wethenshappen. Amsterdam. Proc. Lec. Sci. 28, 432-442.

Horton J.D. (1971). Ontogeny of the immune system in amphibians. Am. Zool. 11, 219-228.

Horton J., Horton T., Ritchie P., Gravenor I., Gartland L. and Cooper M. (1994). Use of monoclonal antibodies to demonstrate absence of T cell development in early thymectomized *Xenopus*. 12th Eur. Congress of Immunology. Abrastract WO9-12, 68.

Ishiguro A., Kobayashi K., Suzuki M., Titani K., Tomonaga S. and Kurosawa Y. (1992). Isolation of a hagfish gene that encodes a complement component. EMBO J. 11, 829-837.

Johnston M.L.R. (1973). Perivascular lymphoid tissue associated with the auxiliar lymph sinus and lateral vein of *Gehyra variegata* (Reptilia: Gekkonidae). J. Morphol. 139, 431-438.

Kampmeier O.F. (1969). Evolution and comparative morphology of the lymphatic system. Charles C.T. (ed). Springfield. Illinois.

Kent S.P., Evans S.E. and Attleberger M.H. (1964). Comparative immunology: lymph nodes in the amphibian *Bufo marinus*. Proc. Soc. Exp. Biol. Med. 116, 456-459.

Kilarski W. and Plytycz B. (1981).The presence of plasma cells in the lamprey (Agnatha). Dev. Comp. Immunol. 5, 361-366.

Kotani M. (1959). Lymphgefasse, Lymphatische apparate und extravasculare. Safbahnen der Schlange (Elaphe quadrivirgata, Boie). Acta Sch. Med. Univ. Kyoto, Japan 36, 121-171.

Kroese F.G.M. and van Rooijen N. (1983). Antigen trapping in the spleen of the turtle *Chrysemys scripta elegans*. Immunology 49, 61-68.

Kroese F.G.M., Leceta J., Deep E.A., Herraez M.P., Nieuwenhuis P. and Zapata A. (1985). Dendritic immune complex trapping cells in the spleen of the snake *Phython reticulatus*. Dev. Comp. Immunol. 9, 641-652.

Kronenberg M., Brines R. and Kaufman J. (1994). MHC evolution: a long term investment in defense. Immunol. Today 15, 4-6.

Lamers C.H.J. (1985). The reaction of immune system of fish to vaccination. Ph D. Thesis. University of Wageningen. Netherlands.

Leceta J. and Zapata A.G. (1991). White pulp compartments in the spleen of the turtle, *Mauremys caspica*. A light-microscopic, electron-microscopic, and immuno-histochemical study. Cell Tissue Res. 266, 605-613.

Le Douarin N. (1966). L'hematopoïese dans les formes embryonaires et jeunes des vertebrés. L'Annee Biol. 5, 105-171.

Litman G.W., Rast J.P., Hulst M.A., Litman R.T., Shamblott M.J., Haire R.N., Hinds-Frey K.R., Buell R.D., Margittai M., Ohta Y., Zilch A.C., Good R.A. and Amemiya C.T. (1992). Evolutionary origins of immunoglobulin gene diversity. In: Progress in immunology VIII. Gergely J., Benczur M., Erdei A., Falus A., Füst G.Y., Medgyesi G., Petranyi G.Y. and Rajnavölgyi E. (eds). Springer-Verlag. Budapest. pp 107-114.

Lloyd-Evans P. (1993). Development of the lymphomyeloid system in the dogfish, *Scyliorhinus canicula*. Dev. Comp. Immunol. 17, 501-514.

Lobb C.J. and Clem L.W. (1981). The metabolic relationships of the immunoglobulins in the fish serum, cutaneous mucus and bile. J. Immunol. 127, 1525-1529.

Manning M.J. and Horton J.D. (1969). Histogenesis of lymphoid organs in larvae of the South Africa clawed toad, *Xenopus laevis* (Dardin). J. Embryol. Exp. Morphol. 22, 265-277.

Manning M.J. and Horton J.D. (1982). RES structure and function of the Amphibia. In: The reticuloendothelial system. A comprehensive treatise. Vol. 3. Phylogeny and ontogeny. Cohen H. and Sigel M. (eds). Plenum Press. New York. pp 423-459.

Mattisson A. and Fange R. (1986). The cellular structure of lympho-myeloid tissues in *Chimaera monstrosa* (Pisces, Holocephali). Biol. Bull. 171, 660-671.

Mattisson A., Fange R. and Zapata A.G. (1990). Histology and ultrastructure of the cranial lympho-haemopoietic tissue in *Chimaera monstrosa* (Pisces, Holocephali). Acta Zool. Stockh. 71. 97-106.

Metcalf D. (1992). The hemopoietic regulators: an embarrassment of riches. BioEssays 14, 799-805.

Muthukkaruppan V.R., Borysenko M. and El Ridi R. (1982). RES structure and function of the Reptilia. In: The reticuloendothelial system. A comprehensive treatise. Vol. 3. Phylogeny and ontogeny. Cohen H. and Sigel M. (eds). Plenum Press. New York. pp 461-508.

Nahm M.H., Kroese F.G.M. and Hoffmann J.W. (1992). The evolution of immune memory and germinal centers. Immunol. Today 13, 438-440.

Nielsen M.H., Jensen H., Braendstrup O. and Wenderlin O. (1974). Macrophage-lymphocyte clusters in the immune response to soluble protein antigen in vitro. II. Ultrastructure of clusters formed during the early response. J. Exp. Med. 140, 1260-1272.

Nossal G.J.V. (1994). Differentiation of the secondary B-lymphocyte repertoire: The germinal center reaction. Immunol. Rev. 137, 173-183.

Page M. and Rowley A.F. (1982). A morphological study of pharingeal lymphoid accumulations in larval lampreys. Dev. Comp. Immunol. Suppl. 2, 35-40.

Papermaster B.W., Condie R.M., Finstad J. and Good R.A. (1964). Phylogenetic development of adaptative immunologic responsiveness in vertebrates. J. Exp. Med. 119, 105-130.

Percy Lord R. and Potter I.C. (1977). Changes in haemopoietic sites during metamorphosis of the lampreys *Lampetra Fluviatilis* and *Lampetra planeri*. J. Zool. Lond. 183, 111-123.

Picker L.J. and Siegelman M.H. (1993). Lymphoid tissues and organs. In: Fundamental immunology. Third ed. Paul W.E. (ed.) Raven Press. New York. pp 145-197.

Pontius H. and Ambrosius H. (1971). Lymphozytares system und immunantwort bei niederen wirbeltieren. Folia Haematol. 96, 333-341.

Pulsford A., Fänge R. and Morrow W.J.W. (1982). Cell types and interactions in the spleen of the dogfish *Scyliorhinus canicula*. An electron microscopic study. J. Fish. Biol. 21, 649-662.

Raison R.L., Hook J., Coverley J., Newton R., Geczy C. and Raftos D. (1994). Humoral immunity in the Agnatha: The role of complement-like proteins. Dev. Comp. Immunol. 18 (Suppl 1), 577 (Abstract).

Riviere H.B., Cooper E.L., Reddy A.L. and Hildemann W.H. (1975). In search of the hagfish thymus. Am. Zool. 15, 39-49.

Sano Y. and Imai C. (1961). Über ein blutbildendes Organ in der sella Turcica des Reisensalamanders *(Megalobatrachus japonicus)*. Z. Zellfors. 53, 471-480.

Scharrer E. (1944). The histology of the meningeal myeloid tissue in the ganoids Amia and Lepisosteus. Anat. Rec. 88, 291-310.

Tanaka Y., Saito Y. and Gotoh H. (1981). Vascular architecture and intestinal hematopoietic nests of two cyclostomes, *Eptatretus burgeri* and ammocoetes of *Entosphenus reissneri:* A comparative morphological study. J. Morphol. 170, 71-93.

Tilney F. (1927). A glandular outgrowth from the roof of the oblongata in *Amia calva*. J. Comp. Neurol. 43, 433-449.

Toivanen P., Naukkarinen A. and Vainio O. (1987). What is the function of bursa of Fabricius? In: Avian immunology. Vol. 1. Toivanen A. and Toivanen P. (eds). Boca Raton. Florida. pp 79-100.

Tomonaga S., Hirokare T., Shinohara H. and Awaya R. (1973). The primitive spleen of the hagfish. Zool. Mag. 82, 215-217.

Tomonaga S., Kobayashi K., Hagiwara K., Yamaguchi K. and Awaya K. (1986). Gut-associated lymphoid tissue in the elasmobranchs. Zool. Sci. 3, 453-458.

Tomonaga S. and Fujii R. (1994). Phylogenetic emergence of immunoglobulin-producing cells. Acta Anat. Nippon. 69, 100 (Abstract).

Torroba M., Chiba A., Vicente A., Varas A., Sacedón R., Jimenez E., Honma Y. and Zapata A.G. (1995). Macrophage-lymphocyte cell clusters in the hypothalamic ventricle of some elasmobranch fish. Ultrastructural analysis and possible functional, significance. Anat. Rec. (in press).

Vialli M. (1932). L'organo linfomieloide mielencefalico dei ganoidi. Arch. Biol. 143, 1-28.

Villena A. and Zapata A.G. (1981). Ultrastructure of the jugular body of *Rana pipiens*. In: Aspects of developmental and comparative immunology. I. Solomon J.B. (ed). Pergamon Press. Oxford. pp 491-492.

Weill J.C. and Reynaud C.A. (1987). The chicken B cell compartment. Science 238, 1094-1098.

Zapata A.G. (1980). Ultrastructure of the elasmobranch lymphoid tissue.

I. Thymus and spleen. Dev. Comp. Immunol. 4, 459-472.

Zapata A.G. and Cooper E.L. (1990). The immune system: Comparative histophysiology. John Wiley and Sons. Chichester.

Zapata A.G., Ardavín C.F., Gomariz R.P. and Leceta J. (1981a). Plasma cells in the ammocoetes of *Petromyzon marinus*. Cell Tissue Res. 221, 203-208.

Zapata A.G., Villena A., Razquin B. and Cooper E.L. (1981b). The jugular body in anuran amphibians: role in immunity. Dev. Comp. Immunol. 5 (Suppl. 1), 129-135.

Zapata A.G., Fange R., Mattisson A. and Villena A. (1984). Plasma cells in adult Atlantic hagfish *Myxine glutinosa*. Cell. Tissue Res. 235, 691-693.