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Review article

Haematopoiesis and haematopoietic organs in fish

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Haematopoiesis is a complex process in which haematopoietic stem cells, the most immature elements of the haematopoietic hierarchy, proliferate and differentiate into various classes of haematopoietic progenitor cells. These progenitor cells have been shown to be able to differentiate into mature blood cells: erythrocytes, lymphocytes, thrombocytes, granulocytes, and monocytes. The pronephros, or head kidney, is a basic organ forming the blood elements, and is also a reservoir of blood cells. Basic haematopoietic structures and mechanisms in fish are similar to those functioning in other vertebrates, and all haematopoietic cell types are very similar to those of mammals.

KEY WORDS: haematopoiesis / head kidney / blood cells / haematopoietic organs

HAEMATOPOIESIS

In fish, as in other vertebrates, the lifespan of peripheral blood cells is limited (depending on the type of blood cells it ranges from a few days to a few months), so these cells are replaced multiple times during an individual's life. The longest living blood cells are erythrocytes. Fischer et al. [20] have shown that the lifespan of these cells in *Carassius auratus langsdorfii* is up to 270 days, while the lifespan of other types of blood cells is much shorter (e.g. 12 days for granulocytes). Despite significant differences in the lifespan of blood cells, the body maintains their number at a more or less stable level owing to efficient haematopoiesis.

Haematopoiesis, also known as haematogenesis, is the process of multiplication, differentiation and maturation of blood cells occurring in the haematopoietic organs, owing to which blood cells are replaced multiple times during an individual's life. The efficiency of haematopoiesis depends on the normal function of haematopoietic stem cells (HSCs), which have the ability to mature and differentiate.

The haematopoietic stem cell is a common precursor for all blood cell lines [18, 19, 31, 32, 33]. As a result of each mitotic division, the HSC reproduces one HSC, while the other resulting cell differentiates into a haematopoietic progenitor cell (HPC), which is the same as the stem cell, with identical morphological, genetic and functional features [28]. The HPC then creates clones of daughter cells of all blood cell lines, from which blood cells of a given type are produced [18, 19, 29, 42].

In vertebrates (including fish), haematopoiesis is regulated by haematopoietic growth factors, which include erythropoietin (Epo), needed for the production of red blood cells and produced during hypoxia; thrombopoietin (Tpo), which stimulates the formation of thrombocytes; G-CSF and GM-CSF, responsible for the growth of granulocytes and monocytes; and interleukins 1–6, which stimulate the proliferation and maturation of lymphocytes [2, 11, 24, 47, 53].

HAEMATOPOIETIC ORGANS

There are a variety of haematopoietic organs in vertebrates. In mammals and birds, haematopoiesis occurs primarily in the bone marrow. In reptiles, the spleen, liver and bone marrow perform this function. Haematopoiesis in caudate amphibians takes place in the kidney, spleen and liver, whereas in frogs bone marrow appears as well [10]. In all vertebrates, haematopoietic organs also include the thymus (the site of T cell maturation).

In most species of bony fish (Teleostei), the dominant haematopoietic organ and reservoir of blood cells is the head kidney (pronephros) [4, 18, 19, 21, 26, 42, 50, 51, 53, 56]. It is a paired organ, completely or partially (depending on the species) separated from the mesonephros. It is located in the anterior part of the abdominal cavity. Wendalaar Bonga [59] and Weyts et al. [60] report that the pronephros combines haematopoietic, immune and endocrine functions (production of blood cells and antibodies as well as cortisol and catecholamines). According to Stosik and Deptuła [56], the pronephros is an analogue of the bone marrow of higher vertebrates and functions as the primary haematopoietic tissue and lymphoid organ in bony fishes.

The head kidney, however, is not the only haematopoietic organ in fish [27, 53]. This function is also performed by the thymus and spleen as well as by gut-associated lymphoid tissue (GALT) and mucosa-associated lymphoid tissue (MALT) [2, 26, 27, 31, 32, 33, 39, 44, 49, 52, 55, 56, 64]. Rombout et al. [51] argue that this function is also performed in part by the mesonephros (the haematopoietic tissue is located between nephrons). Haematopoietic cells in the mesonephros of carp have been observed by Fijan [18, 19], Korwin-Kossakowski and Ostaszewska [37], and Lutnicka and Ludwikowska [40].

In some fish species several haematopoietic organs work simultaneously, while in others only one of them is active [38, 39, 44, 49]. Only the spleen is haematopoietically active in *Salmo trutta*, only the kidney in *Rutilus rutilus*, and both organs in *Perca fluviatilis* [8]. In *Cyprinus carpio* and *Oreochromis niloticus*, the head kidney and the spleen function as the main erythropoietic organs [25, 56].

Most researchers [10, 12, 16, 50] agree that fish lack bone marrow and lymph nodes. However, the literature does contain isolated reports of the presence of bone marrow in these vertebrates. Vermeulen and Kaiser [58] have reported the presence of bone marrow in the vertebrae of *Salmo gairdneri* and consider it a potential source of stem cells and a site of erythropoiesis.

PRIMITIVE AND DEFINITIVE HEMATOPOIESIS

The site of blood cell formation also changes during individual development. In mammals and birds, extraembryonic primitive haematopoiesis takes place in the blood islands of the yolk sac (mainly primitive erythrocytes are formed here). In mammals, the haematopoietic function is taken over by the liver during the foetal period and then, after birth, by the bone marrow. In reptiles, the blood islands of the yolk sac function during the embryonic period, and at the early stage of development the liver and spleen perform haematopoietic functions together with the bone marrow. In amphibians, sites of primitive haematopoiesis are the ventral blood islands, which are the analogue of the above-mentioned yolk sac blood islands, and dorsal lateral plates [9].

The ontogenesis of haematopoietic processes is also fairly well known in some fish species. Długosz [15], who conducted research on *Salmo gairdneri* (Richardson 1836), distinguished two phases of erythropoiesis. The primitive phase occurs extraembryonically in the mesenchyme of the yolk sac walls and in the embryonic mesenchyme, and lasts until the resorption of the yolk sac, while the definitive phase appears gradually from the second day after hatching of the larva and is associated with the start of involvement of the mesonephros in haematopoiesis. The first thrombocytes appeared four weeks after fertilization, while increased leukopoiesis was observed shortly before and immediately after the larvae were hatched.

Davidson and Zon [12] and Chen and Zon [9] observed that in *Danio rerio* (L.) primitive haematopoiesis begins within the embryo, in the anterior lateral mesoderm (ALM), just 10 hours after fertilization. It is mainly myelopoiesis that takes place here, i.e. differentiation of macrophages and granulocytes [3, 7]. Then, 12 hours after fertilization, the primitive stem cells migrate to the intermediate cell mass (ICM), which spreads out on both sides of the body of the embryo [13]. Erythropoiesis takes place here [23, 61]. About 24 hours after fertilization, erythroblasts migrate to the yolk sac, where they begin to circulate and enter the peripheral blood [6, 46, 48]. It is not until four days after fertilization that they transform into embryonic erythrocytes containing haemoglobin [6]. Exceptions are the Antarctic fish *Trematomus bernacchii* (Nototheniidae) and *Chionodraco hamatus* (Channicthyidae), whose erythrocytes do not contain any haemoglobin [43, 50]. The next stage is the posterior blood islands (PBIs), in which primitive haematopoiesis is partially lost.

Definitive haematopoiesis begins two days after fertilization [7, 9] and initially occurs in the ventral wall of the dorsal aorta, in the aorta-gonad-mesonephros (AGM). Next, three distinct haematopoietic organs are formed from the AGM: caudal haematopoietic tissue (CHT), where, as noted by Murayama et al. [46], blood cells differentiate and multiply (and then migrate and settle in the definitive haematopoietic organs), the thymus, and the kidney, which functions as an independent haematopoietic organ from the sixth day [9].

HAEMATOPOIETIC CELL LINES

There is quite a lot of data on the histology and ultrastructure of the haematopoietic tissue of the head kidney in fish [1, 14, 16, 39, 41, 50, 52, 57]. There is less information, however, on the percentages of cells of particular lines in the haematopoietic organs of bony fishes [19, 21, 34, 35, 36, 54, 63]. The available literature indicates that the basic structures of haematopoietic tissue and mechanisms of haematopoiesis in fish are very similar to those of other vertebrates.

All haematopoietic cell lines, including HSCs, are found in the head kidney, although the percentage of particular cell lines and their developmental stages may vary considerably between species [18, 19, 31, 34, 35, 54, 63, 65].

All developmental stages of fish blood cells are very similar to those observed in mammals [9, 18, 19, 32, 33, 34]. An exception is the final stage of maturation of erythrocytes, which do not lose their cell nucleus in fish (so there is no reticulocyte stage). Thrombocytopoiesis in fish differs from this process in mammals; there is no megakaryocyte stage, and the cells released into the peripheral blood remain nucleated [10]. The barrier between the haematopoietic system and the peripheral blood, preventing immature blood cells from entering the blood of fish, is not as restrictive as the marrow barrier in mammals. According to Janicki [28], erythroblasts account for only about 1% in human peripheral blood. In bony fish there are many more, because the cells are released in relatively early stages of development [17, 30] and ultimately do not mature until they reach the blood. The percentage of erythroblasts is 0–12% in *Cyprinus carpio*, [62, 63], 0–4% in *Salmo trutta* [5], 10.6% in *Salmo gairdneri* [30], 23.2–56.5% in *Carassius auratus* [45], and about 6% in *Ictalurus punctatus* [19]. Similarly, in the case of leukocytes, in human blood the last two stages of neutrophilic granulocytes (rod neutrophils and segmented neutrophils) occur almost exclusively, while in fish myelocytes and metamyelocytes are equally numerous.

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