Blood Cells and Blood Cell Development in the Animal Kingdom

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Annu. Rev. Cell Dev. Biol. 2006. 22:677–712

First published online as a Review in Advance on July 12, 2006

The *Annual Review of Cell and Developmental Biology* is online at http://cellbio.annualreviews.org

This article's doi: 10.1146/annurev.cellbio.22.010605.093317

1081-0706/06/1110-0677$20.00

Key Words

hematopoiesis, evolution, hemocyte

Abstract

Recent findings strongly suggest that the molecular pathways involved in the development and function of blood cells are highly conserved among vertebrates and various invertebrate phyla. This has led to a renewed interest regarding homologies between blood cell types and their developmental origin among different animals. One way to address these areas of inquiry is to shed more light on the biology of blood cells in extant invertebrate taxa that have branched off the bilaterian tree in between insects and vertebrates. This review attempts, in a broadly comparative manner, to update the existing literature that deals with early blood cell development. I begin by providing a brief survey of the different types of blood cell lineages among metazoa. There is now good reason to believe that, in vertebrates and invertebrates alike, blood cell lineages diverge from a common type of progenitor cell, the hemocytoblast. I give a synopsis of the origin and determination of the hematocytoblast, beginning with a look at the hematopoietic organs that house hemocytoblasts in adult animals, followed by a more detailed overview of the embryonic development of the hematopoietic organ. Finally, I compare the process of blood lineage diversification in vertebrates and Drosophila.

Contents

BLOOD CELL CLASSIFICATION

Blood Cells in Vertebrates

Vertebrates possess several highly specialized cell types involved in gas transport (red blood cells, or erythrocytes), blood clotting (thrombocytes), and immune response/tissue repair (white blood cells, or leukocytes) (Bessis 1973, Tanaka & Goodman 1972). Erythrocytes and thrombocytes are small, highly specialized cells that, in mammals, have lost their nucleus (**Figure 1*e***). In lower vertebrates (e.g., agnatha, teleosts), red blood cells and thrombocytes retain a nucleus. Leukocytes fall into numerous morphological and functional classes. One distinguishes granulocytes (polymorphonuclear leukocytes) from mononuclear leukocytes (also called agranulocytes). Granulocytes have a segmented nucleus and are packed with granules (lysosomes) filled with a variety of enzymes involved in the attack and digestion of bacteria and other pathogens invading the body (**Figure 1*c***). Mononuclear leukocytes fall into two groups: monocytes (**Figure 1*b***) and lymphocytes (**Figure 1*d***). Like granulocytes, monocytes invade the tissue at sites of infection. In the tissue, they undergo further differentiation into macrophages (histiocytes), which divide and multiply at the sites at which they are needed. Macrophages phagocytose entire cells, such as cells infected by viruses. Besides destroying such cells, macrophages process proteins of the pathogen and present them on their surface in aggregates with major histocompatibility complex (MHC) I/II proteins.[1]

Whereas granulocytes and monocytes are responsible for the tasks of innate immunity, attacking any invading pathogen that presents itself as foreign, lymphocytes (**Figure 1*c***) function in specific immunity. Only few lymphocytes reside within the bloodstream; most are stationary in the various lymphoid organs of the body. B lymphocytes produce antibodies that recognize a specific antigen. This recognition event activates B lymphocytes to proliferate and mass produce antibodies. T lymphocytes carry another type of receptor, the T cell receptor, in their membrane. With these receptors T lymphocytes recognize antigen/MHC complexes presented by macrophages on their surface. This recognition triggers a response in the T lymphocyte that causes the destruction of the antigen-presenting cell. All the aforementioned blood cell types are derived from mitotically active progenitor cells (**Figure 1*a***) found in the hematopoietic tissues (e.g., the bone marrow in mammals). These hemocyte progenitors are released into circulation only infrequently and mostly under pathological conditions.

[1]For a complete list of abbreviations used in this review, please refer to the appendix at the end of the text.

Hemocytes in Animals Without a Coelom or Vascular System

Freely moving cells with structural and functional properties of at least some of the blood cells characterized above for vertebrates can be found in all multicellular animals. In animals that have evolved a true body cavity (coleom) along with a vascular system, these cells are commonly referred to as coelomocytes and/or hemocytes; in animals without a coelom (also known as acoelomates or pseudocoelomates), we speak of amebocytes, interstitial cells, or neoblasts.

Sponges, assumed to represent one of the the most basal clades of multicellular animals (metazoa) (**Figure 2**), have an ectoderm and endoderm as well as a gelatinous matrix, termed the mesoglea, that fills the spaces between these two epithelia (Harrison & de Vos 1991). The mesoglea contains large numbers of motile amebocytes (**Figure 1*u***) that carry out multiple functions. Possibly the most primitive function is digestion: Ciliated endodermal cells (choanocytes) filter food particles into the mesoglea, where they are taken up, broken down, and transported within the body by amebocytes (Van de Vyver 1981). Furthermore, archaeoocytes, which bear structural similarities to blood stem cells in vertebrates, serve as a reservoir of stem cells that produce other cell types, including choanocytes, sclerocytes, and gametes (Mueller et al. 2003, Saller 1988, Tanaka & Watanabe 1984, Van de Vyver 1981, Weissenfels 1981).

Cnidarians and ctenophores (**Figure 2*a***) also have a cell-rich mesoglea that fills the spaces between the ectoderm and endoderm. Cells within the mesoglea, termed interstitial cells (**Figure 1*v*,*w***), are best known for their function as continuously proliferating stem cells (Bode 1996, Martin et al. 1997). Interstitial cells form all tissues in asexually reproducing polyps; in mature animals they replace cells that are lost through wear and tear. Interstitial cells also give rise to the germ line (Miller et al. 2000) and may act as phagocytes (Fautin & Mariscal 1991).

Sponges and coelenterates are traditionally classified as diploblastic (two-germ-layered) animals, although the interstitial cells (and, in ctenophores, a distinct subectodermal muscle layer) could be considered as a primitive middle layer/mesoderm (for a recent discussion of germ layers in coelenterates, see Martindale et al. 2004). In true triploblasts the mesoderm differentiates into numerous different tissues. It is widely held that with the formation of a mesodermal layer in triploblasts the foundation for an enormous diversification in animal body structure was laid. Among the extant triploblasts, several phyla, in particular acoels and other platyhelminths (flatworms), have no coelomic body cavity (**Figure 2*b***). The interior of an acoelomate is filled with a meshwork of mesodermal cells termed parenchyma (Rieger et al. 1991). Fluid-filled clefts within the parenchyma can form a primary body cavity (pseudocoelom) (see Ax 1996, Bartholomaeus 1993).

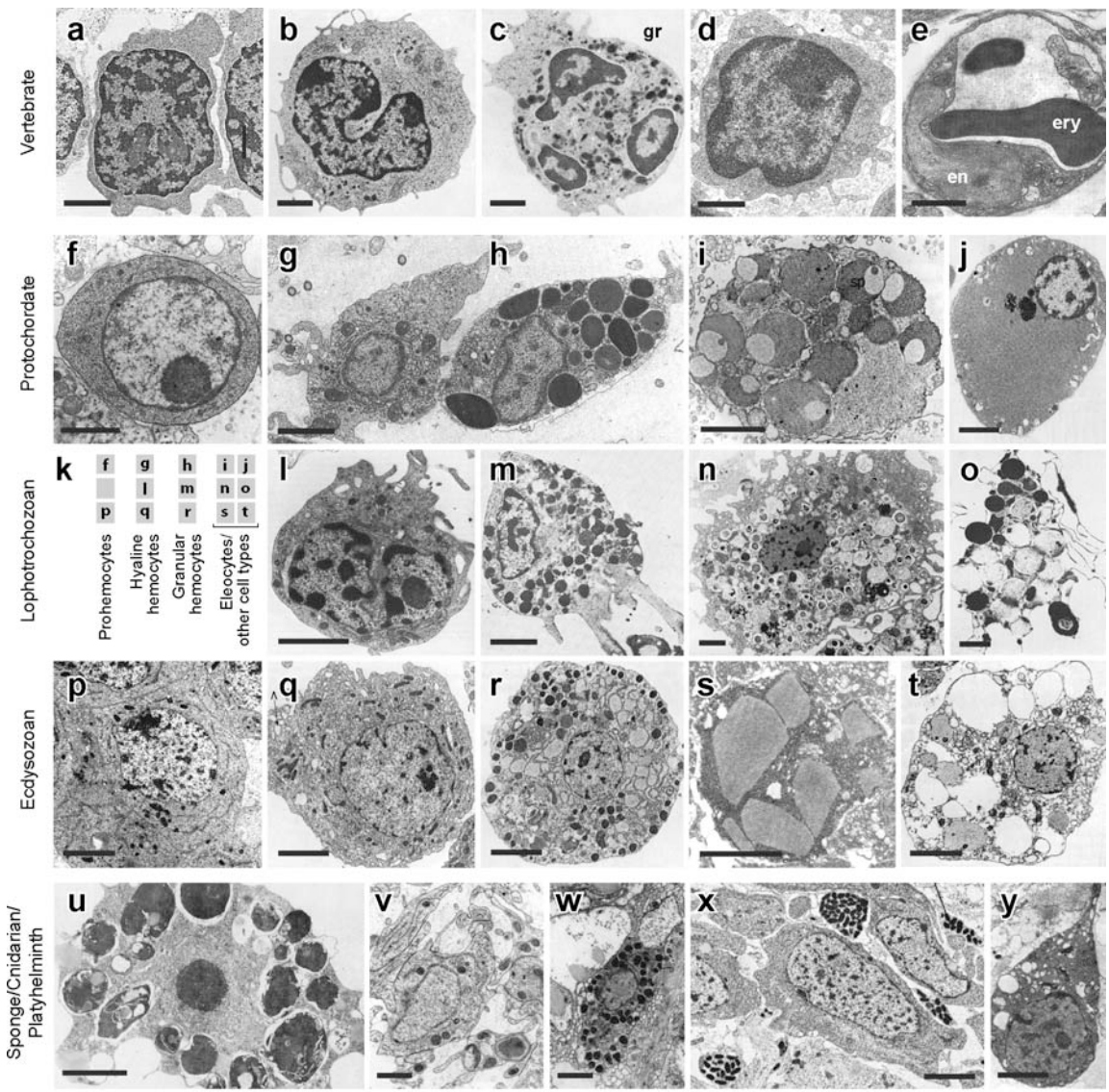
Freely moving cells are observed in the parenchyma of flatworms. These cells are known as neoblasts in the modern literature (**Figure 1*x*,*y***) (Rieger et al. 1991), although early studies that attempted to trace the phylogenetic origin of blood cells applied terms such as lymphocyte and hemocytoblasts (Andrew 1965). Neoblasts, just like interstitial cells in coelenterates, are motile, typically have the appearance of undifferentiated stem cells, and are indeed ultrastructurally comparable with vertebrate hemocytoblasts (Andrew 1965). Neoblasts are able to differentiate into all cell types during normal postembryonic development and regeneration (Ehlers 1985). The advent of molecular markers will help to clarify whether neoblasts in flatworms, or interstitial cells in coelenterates, are truly homologous to hemocytes in more derived animals.

Hemocytes in Invertebrates with Body Cavities and Vascular Systems

Most triploblastic animals possess a secondary body cavity, the coelom. The defining character of the coelom is its

epithelial lining, referred to as the mesothelium, formed by mesodermally derived cells. Typically, the coelom is subdivided into several compartments; in vertebrates, these are the peritoneal cavity, pleural cavity, and pericardial cavity. In the segmented worms (annelids), each segment contains a bilateral pair of coelomata (**Figure 2*c***). In coelomate invertebrates a blood/vascular system is generally well developed. Blood vessels are formed in clefts (sinuses) left open in between the mesothelial walls of the coelomata (Gardiner 1992, Nakao 1974, Ruppert & Carle 1983, Smith 1986). The mesothelial walls of the coelomata are also the site of origin of blood progenitor cells (**Figure 2*c***) (see below).

Classification of invertebrate hemocytes. Hemocytes occur in the vascular lumen and the coelom cavity of all coelomate animals.



Abbreviations: **en**, endothelial cell; **ery**, erythrocyte; **gr**, granulocyte

There is evidence indicating that cells of the coelom (coelomocytes) migrate into the blood vessel lumen to become hemocytes and that cells of the vessel lumen migrate into the coelem (e.g., Cuenot 1897, Valembois 1971). In other words, both cell types would in reality represent a single class of cells. However, this question, relevant in taxa with a closed vascular system, such as annelids or echinoderms, needs to be investigated more carefully. The nomenclature that histologists propose for different hemocyte subclasses is diverse and often idiosyncratic, making it difficult to compare hemocytes in different taxa. For example (as already stated in a comprehensive overview of the classical literature provided in work assembled by Ratcliffe & Rowley 1981), cells that, based on their purported structure and function, appear indistinguishable have been referred to as amebocytes, coelomocytes, lymphocytes, leucocytes, plasmatocytes, and hemocytes, among other terms. Apart from purely semantic factors, there are two other layers of excessive (and probably artificial) complexity in the classification of hemocytes. One is the occurrence of technical artifacts caused by the preparation of hemocytes, which are extremely sensitive to the conditions of fixation and staining. Furthermore, hemocytes undergo directed or cyclic changes in morphology throughout their lifetime, similar to blood cells in vertebrates such as monocytes, which change into histiocytes once they leave the blood vessel lumen. Here I attempt to provide a simplified classification scheme of hemocytes that can accommodate the multitude of blood cell types described for invertebrates. This scheme is based to a large extent on the efforts of Dales & Dixon (1981) (polychaetes), Jamieson (1981) (annelids), Cooper & Stein (1981) (oligochaetes), Cheng (1981) (bivalves), Hoffmann (1969) (insects), Gouin (1970) (insects), Gupta (1979) (insects), Rowley & Ratcliffe (1981) (insects), Smith (1981) (echinoderms), Wright (1981) (Urochordates), and others.

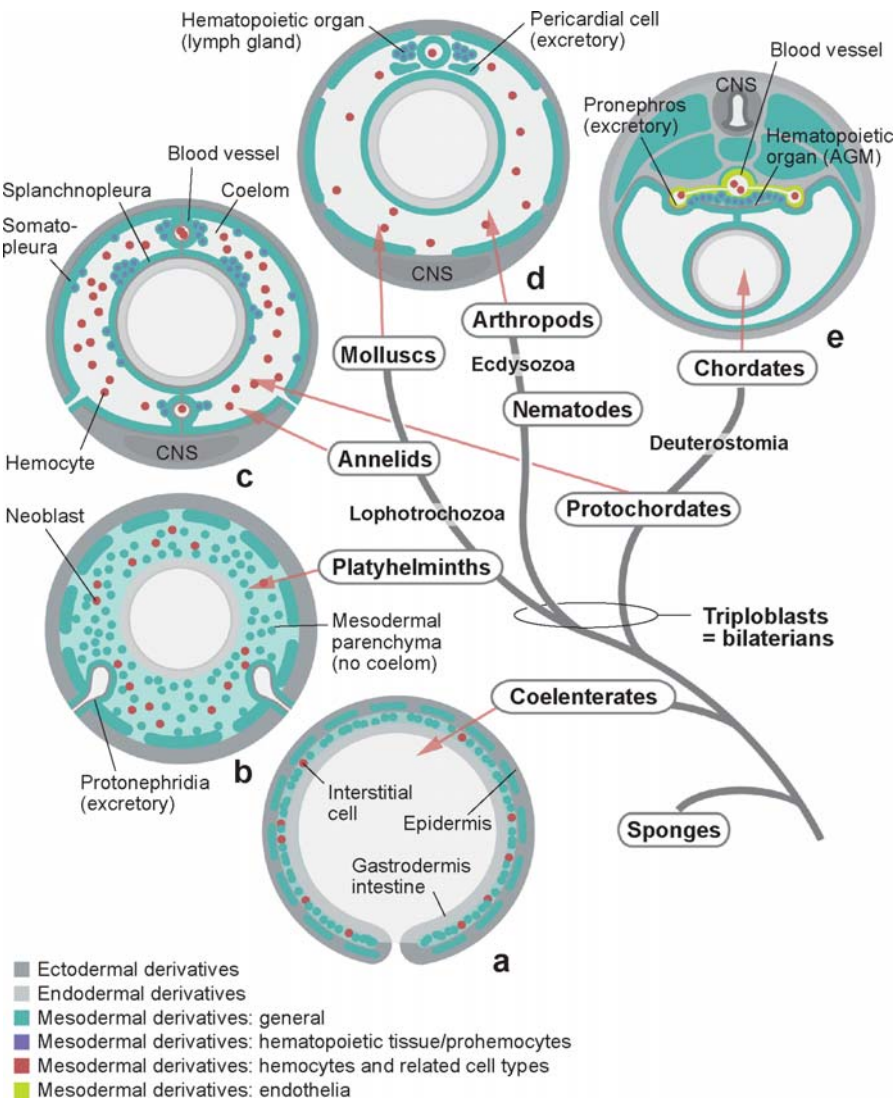
Four major blood cell types—prohemocytes, hyaline hemocytes (plasmatocytes or monocytes), granular hemocytes (granulocytes), and eleocytes (hemocytes with inclusions; also called chloragogenocytes for some taxa)—have been defined structurally (**Figure 1*k***). Prohemocytes are immature cells that, in all taxa in which blood development has been followed, represent the majority of cells in hematopoietic tissues

Figure 1

Synopsis of major blood cell types. Blood cell types found in coelomate invertebrates (*second through fourth rows*) are ordered in a manner illustrated in panel *k*: Prohemocytes are shown in the first column (*f*, *p*), hyaline hemocytes in the second column (*g*, *l*, *q*), granular hemocytes in third column (*h*, *m*, *r*), and eleocytes/other cell types with inclusions in the fourth and fifth columns. Scale bar, 2 μm. (*a–e*) Vertebrate cells. (*a*) Blood progenitor in bone marrow (from Tanaka & Goodman 1972). (*b*) Monocyte (from Bessis 1973). (*c*) Granulocyte (gr) (from Bessis 1973). (*d*) Lymphocyte (from Tanaka & Goodman 1972). (*e*) Erythrocyte (ery) in capillary. From Kierszenbaum 2002. (*f–j*) Protochordate (ascidian). (*f*) Prohemocyte in *Botrylloides leachi* (from Burighel & Cloney 1991). (*g*, *h*) Hyaline and granular hemocyte in *Diplosoma listerianum* (from Burighel & Cloney 1991). (*i*) Spherule cell in *Cucumaria normani* (from Smith 1981). (*j*) Hemoglobin containing hemocyte in *C. normani* (from Smith 1981). (*l–o*) Lophotrochozoan (annelid) cells. (*l*) Lymphocytic coelomocyte (hyaline hemocyte) in *Lumbricus terrestris* (from Linthicum et al. 1977). (*m*) Granular amoebocyte (granular hemocyte) in *Eisenia foetida unicolor* (from Jamieson 1981). (*n*) Eleocyte in *L. terrestris* (from Linthicum et al. 1977). (*o*) Luminiscent coelomocyte in *Diplocardia longa* (from Jamieson 1981). (*p–t*) Ecdysozoan (insect) cells. (*p*) Prohemocyte in *Calliphora* (from Hoffmann et al. 1979). (*q*) Hyaline plasmatocyte in *Galleria mellonella* (from Rowley & Ratcliffe 1981). (*r*) Granular plasmatocyte in *G. mellonella* (from Rowley & Ratcliffe 1981). (*s*) Crystal cell in *Drosophila melanogaster* (from Rizki & Rizki 1984). (*t*) Spherule cell in *G. mellonella* (from Rowley & Ratcliffe 1981). (*u*) Sponge archaeocyte (from Harrison & de Vos 1991). (*v*, *w*) Interstitial cells in the cnidarian *Haliplanella luciae* (from Fautin & Mariscal 1991). (*x*, *y*) Platyhelminth neoblasts (from Ehlers 1985). All figure subparts are used with permission.

(**Figure 1*f*,*p***). They are small, round cells with a relatively large nucleus and scant cytoplasm, resembling blood progenitors in vertebrates. In the peripheral blood/hemolymph of mature invertebrates, prohemocytes typically form but a small percentage of the blood cells. These observations, along with experiments using labeled thymidine to follow blood cell lineages (Shrivastava & Richards 1965), support the view that prohemocytes act as immature blood cell progenitors.

It is widely held that prohemocytes are immature blood precursor cells that differentiate into most, if not all, of the other blood cell types (Cheng 1981, Jamieson 1981, Lebestky et al. 2000, Wigglesworth 1965). The most prevalent type of differentiated blood cells is the hyaline (glassy) hemocytes, or plasmatocytes, which derive their name from the fact that their cytoplasm is relatively smooth and transparent (**Figure 1*g*,*l*,*q***). Plasmatocytes can be best compared with monocytes/macrophages/histiocytes of vertebrates (Evans et al. 2003). They are generally recognized as phagocytotic cells (macrophages) involved in the removal of



apoptotic cells during development as well as in the ingestion or encapsulation of pathogens (innate immune response).

Granular hemocytes (granulocytes) are densely packed with regularly sized granula, which ultrastructurally are electron-dense, enzyme-filled lysosomes (**Figure 1*b*,*m*,*r***). Similar to the situation in vertebrates, neutrophilic, eosinophilic, and basophilic granulocytes were observed in many invertebrate taxa, although the functional significance (if any) of these subclasses is unknown. Granulocytes are involved in developmental and metabolic functions as well as in immune functions, including wound healing, blood clotting, phagocytosis, and encapsulation of pathogens.

Besides plasmatocytes and granulocytes, the blood/hemolymph/coelom compartments of many taxa contain a diverse group of free cells that contain irregularly sized and shaped lipid or crystalline inclusions. There are many different names in use, among them eleocytes (the term used here to denote this cell type), chloragogen cells, vacuolated cells, spherulocytes, adipohemocytes, and oenocytoids (**Figure 1*i*, *j*,*n*,*o*,*s*,*t***).

Red blood cells containing the oxygen carrier, hemoglobin, constitute the numerically most prevalent cell type in vertebrate blood. Hemoglobin and other oxygen-binding proteins, such as hemocyanin, occur in several invertebrate taxa, either dissolved in the blood/hemolymph (in, e.g., nematodes, molluscs, arthropods) (Cowden & Curtis 1981, Sherman 1981, Van de Vyver 1981) or packed into plasmatocytes (in, e.g., some annelids, sipunculids, lophophorates, and echinoderms) (Cooper & Stein 1981, Dybas 1981, Hayward 1981). However, such dedicated oxygen-carrying red blood cells seem to represent the exception among invertebrates.

Comparison of blood cell types in lophotrochozoans, ecdysozoans, and deuterostomes. Freely moving cells that conform to the four classes of hemocytes defined above have been identified in most, if not all, coelomate phyla. Classical studies of lophotrochozoan phyla (**Figure 2*c***) (e.g., molluscs, annelids, sipunculids, echiurids) reveal prohemocytes and hyaline and granular hemocytes next to a wealth of differently structured eleocytes. In many annelid species eleocytes filled with lipid granules form a conspicuous chloragogen tissue surrounding the intestinal wall that may be compared with the liver of vertebrates and the fat body of arthropods. Eleocytes take up, digest, and

Figure 2

Phylogenetic tree of major animal body plans. The tree, at its base, highlights the two major diploblast taxa, sponges and coelenterates. It then branches into the three main clades of triploblastic animals: lophotrochozoa, ecdysozoa, and deuterostomia. Arranged around the tree are schematic cross sections of phyla representing the major body plans. (*a*) Diploblast. Interstitial cells in between endoderm and ectoderm may constitute the origin of the third germ layer (*aqua*); among the motile interstitial cells are some with phagocytic function (*red*). (*b*) Acoelomate triploblast (platyhelminth or flatworm). The space between ectoderm and endoderm is filled with a mesodermal parenchyma that contains motile cells acting as stem cells (neoblasts) as well as phagocytes. (*c*) Coelomate (e.g., annelids, lower deuterostomes). The closed body cavity (coelom) is surrounded by a mesodermally derived epithelium (mesothelium). The outer layer (somatopleura) lines the body wall; the inner layer (splanchnopleura) envelops the inner organs. Blood vessels evolve as clefts in between mesothelia. Mesothelia also produce hemocytes, which spread out in the coelom and blood vessels. (*d*) Myxocoelomate (e.g., molluscs, arthropods). The body cavity (myxocoel) is not lined by a complete mesothelial layer. Reduced mesothelia form blood vessels and sinuses around some organs (e.g., gonads). Hemocyte progenitors are clustered in specialized hematopoietic organs (lymph glands) typically associated with blood vessels. CNS, central nervous system. (*e*) Coelomate (chordates). Complete mesothelium (splanchnopleura and somatopleura) is present. Progenitors of both blood vessels and blood (hemangioblasts) split from the mesothelium in the embryo and form endothelia and specialized hematopoietic organs. AGM, aorta-gonad-mesonephros.

distribute nutrients (Cooper & Stein 1981, Jamieson 1981). Conversely, they may act to store metabolic waste products from the blood/hemolymph and to deliver them to the intestine, where the waste products are excreted. In some taxa (e.g., sipunculids), peculiar motile organules termed ciliated urns populate the coelomic liquid (Dybas 1981). They consist of a pair of densely ciliated cells surrounded by a belt of lobe cells and are active in secreting extracellular matrix (ECM) as well as phagocytosing pathogens.

Blood cell types and their functions have been carefully analyzed for numerous insects, crustaceans, and other arthropod taxa representing the ecdysozoan clade (**Figure 2*d***). Prohemocytes, plasmatocytes, and granulocytes resembling their annelid counterparts form the majority of circulating blood cells. Spherulocytes or adipohemocytes, resembling eleocytes in annelids, are blood cells with variably sized and shaped inclusions. Several authors have claimed that these cells represent late stages in the differentiative pathway of phagocytic plasmatocytes (reviewed in Rowley & Ratcliffe 1981; among the more recent studies of insect blood cell types that confirm the previous classification are Beetz et al. 2004, Brehelin & Zachary 1986, Butt & Shields 1996, Chiang et al. 1988, Essaway et al. 1985, Giulianini et al. 2003, and Pelc 1986). One type of circulating blood cell that appears to be unique to arthropods are the oenocytoids. These are large, oval cells with a cytoplasm devoid of normal organelles except for fibrous agglomerates of crystalline material and/or microtubules. The so-called crystal cells of *Drosophila* (**Figure 1*s***) are likely to correspond to the oenoytoid defined for other arthropods (Brehelin 1982) because neither cell type has regular cytoplasmic organelles. A number of studies have reported the generation of antibodies specifically recognizing individual hemocyte classes or combinations thereof (Beetz et al. 2004, Gardiner & Strand 1999, Mullett et al. 1993). These studies generally confirm the validity of morphological criteria classifying hemocytes in the above defined major classes and may be used in the future to shed more light upon the transitions between different classes during hematopoiesis.

Terrestrial arthropods have evolved a complex system of cellular and humoral factors to cope with tissue injury, wound repair, and the response to parasites and other pathogens. The experimental analysis of these factors, greatly aided by genetic approaches in *Drosophila* (see *Drosophila* section, below), is just beginning. Injuries evoke a clotting response that consists of the aggregation of hemocytes, followed by plama coagulation caused by the release of clotting factors from storage granules in hemocytes (Hoffmann 1995, Kanost et al. 2004, Lavine & Strand 2002, Theopold et al. 2002, Tzou et al. 2002). Foreign bodies (such as the eggs of parasites deposited in the host body cavity) are countered by cellular capsules formed by plasmatocytes. For some arthropods, characteristic cell types and enzyme systems carrying out these immune responses have been described. Coagulocytes may represent a specialized type of plasmatocyte, characterized by characteristic fibrillar/punctate inclusions surrounded by membranes (Goffinet & Gregoire 1975, Hoffmann 1969). These inclusions are discharged during coagulation. Regular plasmatocytes or granulocytes may build the cellular clot in other taxa. An enzymatic-cascade-activating phenol oxidase is involved in clotting as well as the encapsulation of pathogens. Phenol oxidase, along with granulocytes and oenocytoids/crystal cells, is detected in the aforementioned coagulocytes (Ashida et al. 1988, Iwama & Ashida 1986, Rowley & Ratcliffe 1981, Wigglesworth 1988).

Thymidin labeling was used to determine the relationship between different structural types of hemocytes in arthropods. According to several studies (reviewed in Gouin 1970), prohemocytes first differentiate into plasmatocytes, which then become adipohemocytes. Granulocytes and oenocytoids probably descend from plasmatocytes as well (Hoffmann

1969). Finally, spherulocytes represent the final (degenerative) stage in the development of oenocytoids. Wigglesworth (1965) concurs with the lineage of prohemocyte, plasmatocyte/granulocyte, oenocytoid, spherulocyte. Likewise, recent studies in *Drosophila* clearly show that all differentiated blood cell types derive from prohemocytes (Evans et al. 2003, Meister & Lagueux 2003, Schulz & Fossett 2005; see *Drosophila* section, below).

In the lower deuterostome phyla (**Figure 2**) (e.g., echinoderms, hemichordates, urochordates) as well as in the cephalochordates—the sister taxon of vertebrates—hemocyte classes with characteristics very similar to those of lophotrochozoans and ecdysozoans have been reported (**Figure 1*f–j***) (Rhodes & Ratcliffe 1983, Smith 1981, Wright 1981). Typical granulocytes appear absent from echinoderms, although hemocytes with large, polymorphic inclusions (vacuolated cells) have been described. Urochordates (protochordates), the closest relatives to the chordate phylum, possess prohemocytes, hyaline and granular hemocytes, and a variety of other blood cell types containing inclusions. Cephalochordates are poor in their diversity of blood cells. Granular hemocytes and macrophages have been described in the coleom and vascular lumen of *Amphioxus* (Rhodes et al. 1982). It appears, thus, that the astounding diversification of blood cell types that we encounter in all extant vertebrates took place during the early evolution of this taxon.

ONTOGENY AND PHYLOGENY OF BLOOD CELL PROGENITORS (HEMOCYTOBLASTS)

Blood cells are produced continuously in the mature animal. More than most other cell types, their rate of formation fluctuates. Responsible for many metabolic functions and, in particular, the immune response, blood cell formation has to be upregulated on demand upon injuries and pathogen invasion. Although mitotic division of mature hemocytes (e.g., plasmatocytes) has been observed in most animal taxa, the majority of blood cells appear to derive from self-renewing populations of multipotent stem cells [termed hemocytoblast, or hemocyte stem cell (HSC), in vertebrates and hemocyte progenitors in invertebrates] that are housed in specialized hematopietic organs. This section provides a brief comparative overview of the structure of these hematopoietic organs. Subsequently, developmental and molecular aspects of early hematopoiesis (that is, the formation of hemocytoblasts) are discussed for vertebrates and *Drosophila*, the one invertebrate for which recent studies have shed light on blood development.

Structure of Hematopoietic Organs

Hematopoietic organs have been described for all major taxa of coelomate animals. In invertebrates, they are typically mesenchymal or gland-like structures attached to the lining of blood vessels and/or the coelomic cavity. In the simplest scenario (e.g., some polychaetes), specialized domains within the mesothelium show higher rates of proliferation and bud off hemocytes into the lumen of the coelom or blood vessels (**Figure 3**) (Dales 1961, Dales & Pell 1970, Eckelbarger 1976; reviewed in Dales & Dixon 1981). The same origin of hemocytes from mesothelial cells lining the coelom/blood vessels has been observed in other invertebrates, including lower deuterostomes (Hausmann 1931, Hetzel 1965). This hematopoietic mechanism may give us a glimpse into the origin of the close ontogenetic relationship between hemocytes and vascular cells. Thus, for *Drosophila* a common progenitor (prior to its last round of division) gives rise to both vascular and hemocyte progenitors (Mandal et al. 2004), and the same seems highly likely in vertebrates as well (Choi et al. 1998, Fehling et al. 2003). In vertebrates, suspected common progenitors of endothelial and blood progenitors were termed hemangioblasts (Murray

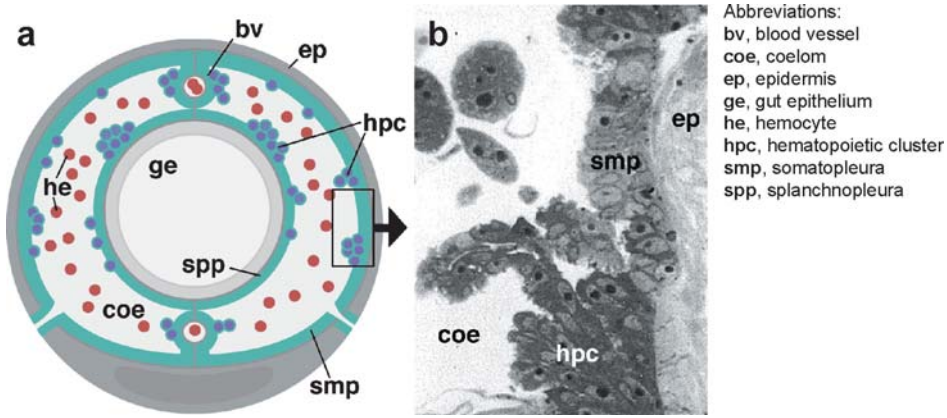


Figure 3

Origin of hematopoietic tissues. (*a*) Schematic cross section of a typical coelomate invertebrate blood vessel. In many coelomate invertebrates (such as polychaete annelids, represented here), hematopoietic cells form clusters (hpc) within the somatopleura (smp), splanchnopleura (spp), and blood vessel (bv) wall. (*b*) Histological section of somatopleura of the polychaete *Nicolea zostericola*, depicting hematopoietic clusters (hpc) that bud off hemocytes into the coelom (coe) (from Eckelbarger 1976, with permission).

1932). It is reasonable to assume that phylogenetically, hemangioblasts originated as bi- or pluripotent cells populating the coelomic epithelium of a primitive ancestral invertebrate. More detailed studies of the origin of hemocytes in polychaetes and other simple coelomates, which may have retained primitive aspects of the bilaterian ancestor, may be highly informative in regard to the evolutionary origin of hematopoietic organs.

We encounter a more complex hematopoietic mechanism in oligochaetes, some molluscs, arthropods, and ascidians. In these taxa, hematopoietic stem cells moved out of the mesothelium and coalesced in compact hematopoietic organs called lymphoid organs or lymph glands (**Figure 4**). Lymph glands are typically attached to the coelomic wall or large blood vessels. In oligochaetes and insects, lymph glands form a metameric pattern of encapsulated organs flanking the wall of the dorsal blood vessel (**Figure 4*b***) (Cuenot 1897, Hoffmann et al. 1979, Kindred 1929). These invertebrate lymph glands consist of spongy mesenchymal masses of cells, many of which are mitotically active and give birth to various types of hemocytes that initially fill the lacunae within the center of the gland before moving out into the lumen of the coelom or hemolymph space (**Figure 4*c***). Similar structures have also been described for mollusks [e.g., the amebocyte-producing organ in gastropods (Jeong et al. 1983), the "white glands" of cephalopods located close to the eye (Cowden & Curtis 1981)] and crustaceans (the lymph glands along the vessels near the eyes and proximal appendages; reviewed in Bauchau 1981). In ascidians, lymph glands (described as blood-forming nodules) are grouped around the transverse bars of the pharyngeal basket, generally assumed to represent the phylogenetic forebear of the gill apparatus of vertebrates (Wright 1981).

Invertebrate lymph glands contain mostly proliferating prohemocytes but also contain differentiated blood cells such as plasmatocytes. A zonation is often visible (**Figure 4*d–b***), such that the undifferentiated hemocyte progenitors clump together, and more differentiated hemocytes form an outer layer around them [Hoffmann et al. 1979, Jung et al. 2005, Lanot et al. 2001, Nardi et al. 2003, and Shrestha & Gateff 1982 (insects); Cowden & Curtis 1981 (cephalopods); Ermak 1976 (ascidians)]. A prominent stroma, akin to the network of fibroblasts and capillaries found in the

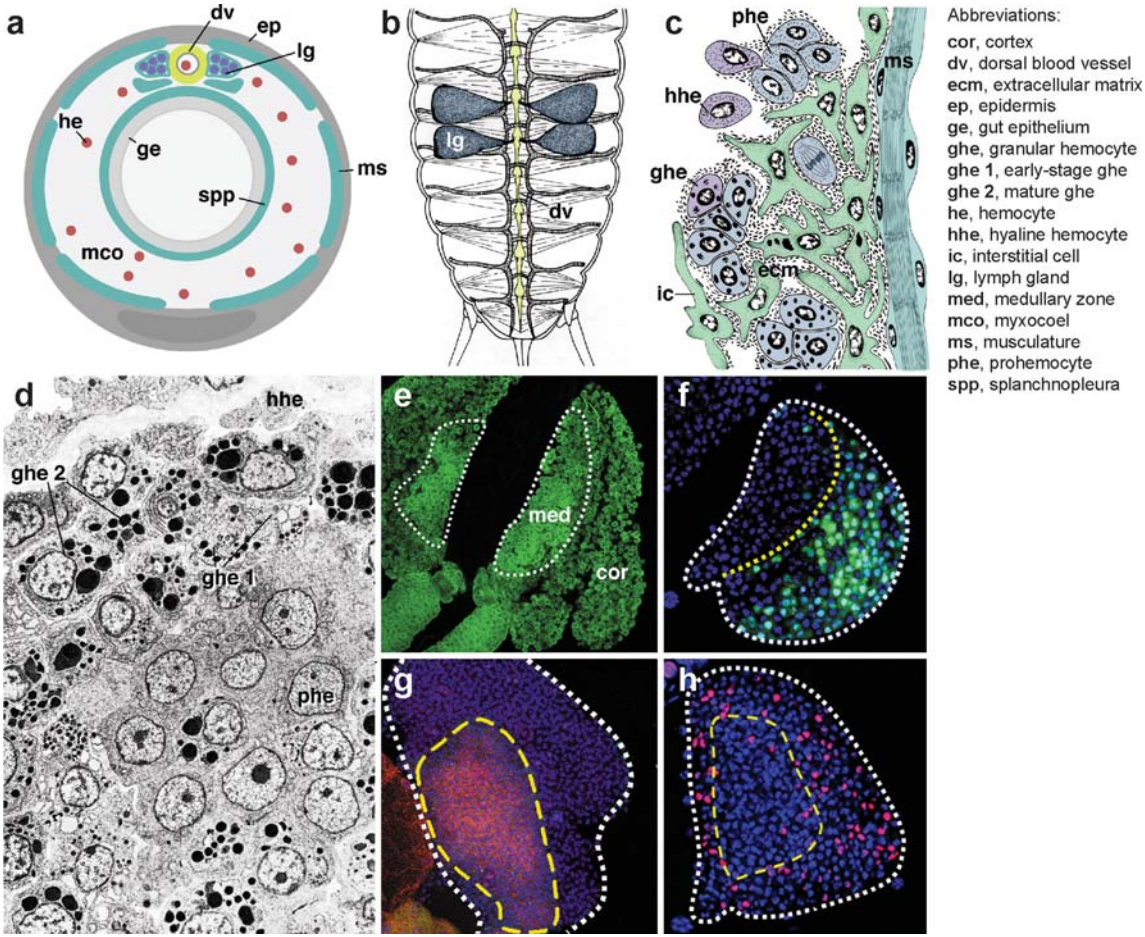


Figure 4

Hematopoietic organs (lymph glands) in invertebrates. (*a*) Schematic cross section of arthropod showing a close association of lymph gland (lg) and dorsal blood vessel (dv). (*b*) Schematic dorsal view of insect, showing dorsal blood vessel (dv) and attached bilateral, segmentally arranged lymph glands (lg) (after Hoffmann et al. 1979). The location of lymph glands along the anteroposterior axis varies among insects: In cricket, shown here, lymph glands occur in the abdomen; in *Drosophila*, they are found around the anterior portion of the dorsal vessel in the thorax. (*c*) Line drawing of section of insect lymph gland (after Hoffmann et al. 1979). Attached to dorsal muscles (ms) and dorsal vessel (*not shown*), the lymph gland consists of immature prohemocytes (phe) that give rise to different lineages of hemocytes (hhe, hyaline hemocytes; ghe, granular hemocytes). Mesodermal interstitial cells (ic) may represent stroma or the early stage of blood progenitors. (*d*) Electron micrograph of hematopoietic organ of ascidian (from Ermak 1976, with permission). Note the layered organization, with prohemocytes (phe) at the lower right, early stages of granular hemocytes (ghe 1) in the center, and more mature granular cells (ghe 2) at the upper left. (*e–h*) Confocal images of *Drosophila* larval lymph gland (from Jung et al. 2005, with permission). (*e*) Global nuclear staining, depicting tightly clustered immature prohemocytes in the center (med, medullary zone), surrounded by more loosely packed differentiating hemocytes (cor, cortex). (*f*) Hemocyte differentiation marker (P1; *green*) labels exclusively cortex of lymph gland. (*g*) Adhesion molecule DE-cadherin (*red*) is expressed at high levels in the medullary zone. (*h*) Proliferation (BrdU; *red*) is more pronounced in the cortex.

hematopietic tissue of vertebrates, is missing in invertebrates. In some instances, scattered muscle cells or undifferentiated mesenchymal cells penetrate the lymph glands; a basement typically covers the gland at its outer surface (Ermak 1976, Hoffmann et al. 1979).

The bone marrow is the hematopoietic organ in all vertebrates but fishes, in which hematopoiesis occurs in the kidney. Osteoblasts (bone-forming cells) form a layer, termed endosteum, at the interface between the mineralized bone and the bone marrow contained within its center. Blood vessels, capillaries, and wide, endothelium-bound spaces called venous sinuses branch throughout the bone marrow (**Figure 5*a–c***). Endothelial cells, osteoblasts, and stromal (also called reticular) cells that crisscross the space between vessels and endosteum form a three-dimensional scaffold that houses clusters of blood-forming cells (Greep & Weiss 1973; Kierszenbaum 2002). This scaffold provides a complex microenvironment that, by means of cell-membrane-bound and secreted factors, controls the determination and proliferation of the different blood cell lineages. The multipotent hematopoietic stem cells (HSCs) that seed the bone marrow in the late embryo settle at the outer (subendosteal) layer in contact with the osteoblasts (**Figure 5*d–f***); these latter cells, forming the stem cell niche of the bone marrow, emit signals that maintain HSCs in their noncommitted stem cell mode (Arai et al. 2004, Moore 2004, Taichman 2005, Zhang et al. 2003, Zhu & Emerson 2004).

HSC-derived cells that lose contact with the osteoblast layer progress toward the next stage, that of a committed progenitor for lymphoid cells, red blood cells, thrombocytes, (neutrophile) granulocyte/monocytes, basophile granulocytes, or eosinophile granulocytes. These different progenitors are then found nearer the center of the bone marrow, where they proliferate and form growing colonies of maturing blood cells (**Figure 5*b*,*c***). Once matured, blood cells become capable of crossing the endothelium into the bloodstream. Lymphoid progenitors leave the bone marrow at an immature state and populate the thymus and lymphoid organs.

Early Hematopoiesis in Vertebrates

From the bloodstream, HSCs arrive in the bone marrow and other blood-forming organs during the late embryonic stages. This subsection discusses the origin and specification process of HSCs in the early vertebrate embryo. Vertebrate hematopoiesis traditionally has been divided into an early (primitive) phase and a late, or definitive, phase. Primitive hematopoiesis produces only a restricted range of blood cell types, including primitive (nucleated) red blood cells, granulocytes, and macrophages. Primitive blood cells, which populate the early embryo, have properties that diverge from those of their definitive counterparts. Recent studies (for excellent reviews, see, among others, Baron 2003, Bertrand et al. 2005, Crosier et al. 2002, Davidson & Zon 2004, Dieterlen-Lievre et al. 2005, Eichmann et al. 1997, Galloway & Zon 2003, Tavian & Peault 2005, and Yoder 2002), employing molecular markers and a genetic approach, have elucidated the pattern of hematopoiesis in several vertebrate species. These studies have shed light on how primitive and definitive hematopoiesis hang together, which embryonic tissue gives rise to HSCs, how these cells relate to the progenitors of other tissues, and what molecular mechanisms are required for these cells' specification.

In zebrafish, primitive and definitive hemangioblasts, as well as nephrocyte progenitors, overlap in a narrow beltlike region flanking the somites that is termed the anterior and posterior lateral mesoderm (ALM and PLM, respectively) (**Figure 6*a*,*d***) (Gering et al. 1998). In this region, markers for hemangioblasts [e.g., the bHLH transcription factor Scl and the vascular endothelial growth factor (VEGF) receptor Flk1] and markers for nephrocytes (e.g., the transcription factor Pax 2.1) initially overlap fully. Subsequently, cells of the lateral mesoderm

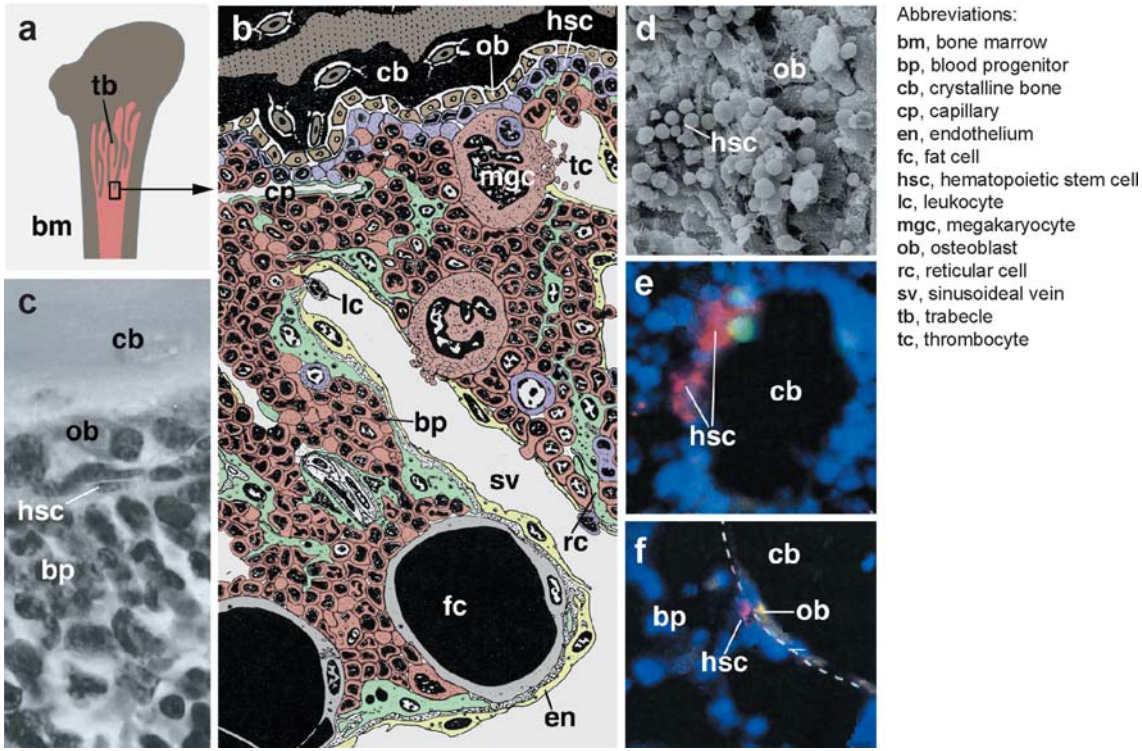


Figure 5

Hematopoiesis in vertebrate bone marrow. (*a*) Marrow (bm) lies in the center of long bones. Branched processes of bone, called trabecles (tb), protrude into the marrow and greatly enlarge the surface at which bone and marrow contact each other. (*b*) Colored line drawing of bone marrow section (after Greep & Weiss 1973). Trabecle formed by crystalline bone (cb) is seen at the top of the picture. Bone-forming cells (ob, osteoblasts) are located within the bone matrix but also form an epithelial layer (endosteum) at inner bone surface. The center of the bone marrow is formed by endothelially (en) lined capillaries (cp) and sinusoideal veins (sv) that are connected with each other and the bone surface by a meshwork of reticular cells (rc; *green*). Reticular cells form the stroma of the bone marrow, which contains proliferating and differentiating clusters of hematopoietic stem cells (HSCs) (denoted in figure by hsc; *violet*) and blood progenitors (bp; *brown*) as well as postmitotic maturing blood cells. HSCs are enriched in the zone that contacts osteoblasts; this zone is believed to constitute the HSC niche. Differentiating blood cells are seen to enter the circulation; these include thrombocytes (tc), produced by the fragmentation of large megakaryocytes (mgc), and leukocytes (lc). (*c*) Histological section of human bone marrow showing the interface of bone matrix (cb), covered by osteoblasts (ob), with HSCs and blood progenitors (bp) (from Balduino et al. 2005, with permission). (*d*) Scanning electron micrograph of inner bone surface with osteoblasts (ob) and HSCs (from Balduino et al. 2005, with permission). (*e*, *f*) Confocal section of embryonic mouse bone marrow. Trabecle of bone (cb) is surrounded by osteoblasts (ob) and bone marrow (*blue*; global nuclear labeling). Panels *e* and *f* from Zhang et al. 2003, with permission. (*e*) HSCs are labeled in pink by stem cell marker Sca; the green label represents BrdU-positive proliferating cells. (*f*) Contact of osteoblasts (ob), marked by expression of N-cadherin (*yellow*), with HSCs (*pink*; labeled with BrdU).

destined to form the blood/vascular system migrate medially and dorsally, whereas nephrocyte precursors remain closer to the lateral surface (**Figure 6*b***). At this stage, Pax2.1 becomes restricted to nephrocyte precursors, and Scl to blood/vascular precursors. The latter cells end up as an unpaired cluster of cells, termed the intermediate cell mass, in

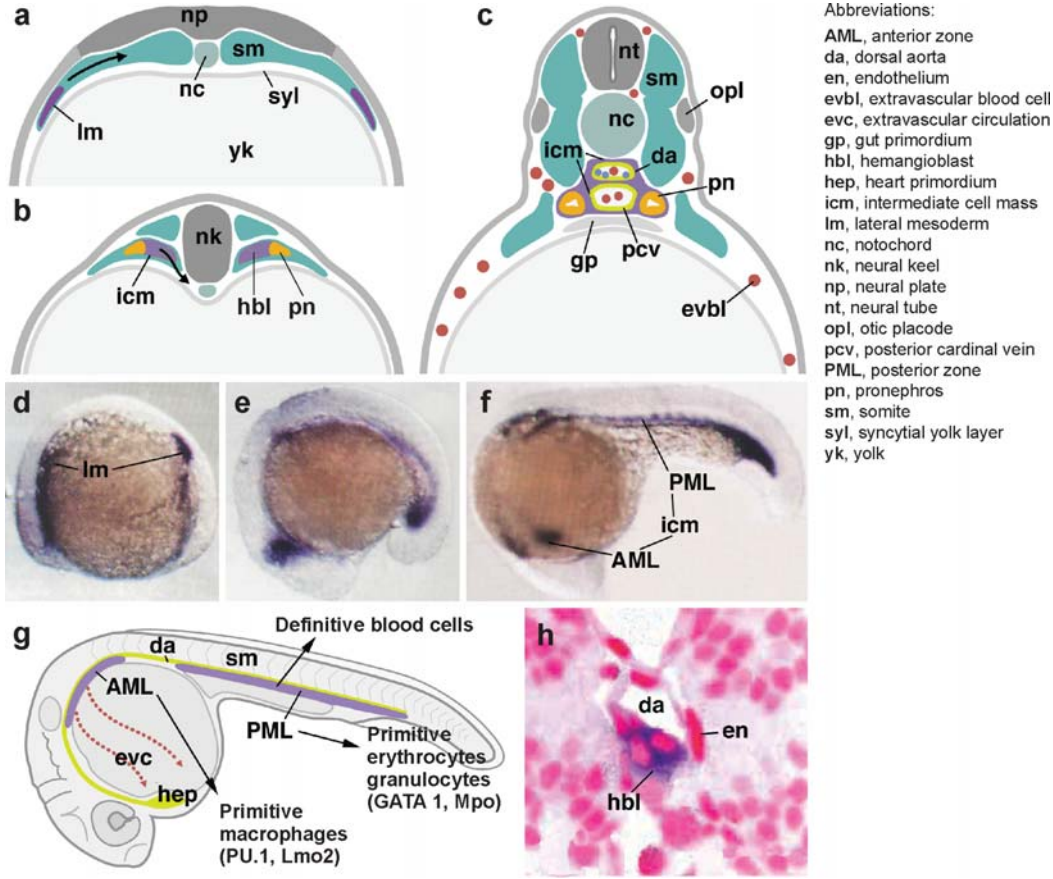


Figure 6

Early hematopoiesis in zebrafish. (*a–c*) Schematic cross sections of zebrafish embryo (*a*) at the end of gastrulation (10 h), (*b*) after neurulation (10-somite stage, 15 h), and (*c*) during the late stage of organogenesis (24 h). In the early embryo (*a*), the lateral mesoderm (lm) represents a population of pluripotent cells that contains all progenitors of blood, blood vessels, and excretory cells. These different cell fates sort out from each other as the lateral mesoderm migrates medially (*b*) (nephrocytes forming pronephros, *orange*; hemangioblasts, *violet*). (*c*) In the late embryo, descendants of the lateral mesoderm form the intermediate cell mass (icm), which is located in between the notochord (nc) and the endodermal gut primordium (gp). The intermediate cell mass contains hemangioblasts that differentiate into blood vessels (da, dorsal aorta; pcv, posterior cardinal vein) and hematopoietic stem cells (HSCs). Primitive blood cells migrate out from the intermediate cell mass before vessels have formed (evbl, extravascular blood cells). HSCs that give rise to definitive blood cells form in and around the dorsal aorta (see also panel *h*). (*d–f*) Lateral views of wholemounts of zebrafish embryos at (*d*) 11 h, (*e*) 15 h, and (*f*) 24 h that are labeled with a probe against the hemangioblast marker Scl. This marker is expressed in lateral mesoderm (lm) and the intermediate cell mass (icm) derived from it. An anterior zone (AML) is distinguished from a posterior zone (PML). Panels *d–f* from Gering et al. 1998, with permission. (*g*) Schematic lateral view of 24-h zebrafish embryo depicting vascular cells (hep, heart primordium; da, dorsal aorta) and intermediate cell mass. The AML gives rise to macrophages, which migrate over the yolk sac (evc, extravascular circulation; *dashed arrows*) and populate extravascular spaces of the embryo. The PML produces mostly primitive red blood cells in addition to HSCs of definitive hematopoiesis. (*h*) Micrograph of section of 24-h zebrafish embryo showing dorsal aorta (da). The endothelial wall (en) of this vessel contains hemangioblasts (hbl) labeled with probe against the AML1 gene (from Kalev-Zylinska et al. 2002, with permission).

the midline of the embryo, located in between the notochord and endoderm (**Figure 6*c***) (Al-Adhami & Kunz 1977, Detrich et al. 1995, Gering et al. 1998). Subsequently, the cells of the intermediate cell mass undergo morphogenesis and form the dorsal aorta (dorsally), cardinal vein (ventrally), and hemocyte progenitors (Bennett et al. 2001, Fouquet et al. 1997, Lieschke et al. 2001). Flk1 expression becomes restricted to the vascular cells of the aorta and vein; Scl remains in blood and endothelial cells. Correspondingly, both blood and blood vessels are affected in Scl knockdown experiments (Patterson et al. 2005). The first blood cells that form in the intermediate cell mass differentiate into three different types of blood cells. In the anterior intermediate cell mass, located in the head of the embryo and derived from the ALM, the cells give rise to primitive macrophages expressing GATA2, Lmo2, and Pu1; in the trunk, hemocyte precursors of the intermediate cell mass form primitive erythrocytes (expressing GATA1) and granulocytes (**Figure 6*g***) (Bennett et al. 2001, Davidson & Zon 2004, de Jong & Zon 2005, Herbomel et al. 1999). Primitive hemocytes differentiate before endothelial cells form a closed vascular system. In the absence of blood vessels, hemocytes initially migrate within the mesoderm, spreading out over the yolk sac (extravascular circulation) and throughout the early developing brain (Herbomel et al. 2001), where they also contribute to a primitive form of microglia. After vessels form, primitive hemocytes are found within the bloodstream for a limited period of time.

Definitive hematopoiesis in zebrafish is initiated in the floor of the aorta and the intermediate mesoderm [a spatial pattern closely similar to the aorta-gonad-mesonephros (AGM) defined in mouse; see below]. Definitive HSCs are marked by the renewed expression of the hemocyte determinant AML1 (**Figure 6*b***) (Kalev-Zylinska et al. 2002). Given that the aorta forms part of the intermediate cell mass, in zebrafish the definitive HSCs must have the same origin as primitive hemocytes; the only differences between the two populations are that the former appear later and are self-renewing, whereas the latter differentiate early and have a limited life span. Definitive HSCs are budded off into the lumen of the aorta, through which they migrate to the blood-forming and lymphoid organs.

Definitive and primitive hemangioblasts in amphibians appear to originate from separate regions within the lateral mesoderm. In *Xenopus*, primitive hemangioblasts are located in the ventrolateral wings of the lateral plate mesoderm, forming the so-called ventral blood island (**Figure 7*a*,*d*,*e***) (Walmsley et al. 2002). By contrast, definitive hemangioblasts, visualized at an early stage by their expression of Flk1 or Scl, come from a more dorsal region. From that position they migrate dorsomedially to form the great blood vessels (Cleaver et al. 1997). Nephrocyte progenitors giving rise to the kidneys are even more dorsal, forming a distinct column between the lateral plate and somites that is called the intermediate mesoderm (**Figure 7*c***). The exact relationship of blood progenitors and the vessels needs further elucidation; for example, it is not yet clear whether, as in zebrafish, progenitors of definitive hematopoiesis form part of the endothelium lining the vessels.

In birds and mammals, primitive hemangioblasts are extraembryonic, populating the yolk sac as the so-called blood islands. In chicken, the primordium of the blood islands (BI) is established as a horseshoe-shaped domain formed by mesoderm that ingresses first during gastrulation (**Figure 8*a–d***) (Ferkowicz & Yoder 2005, Minko et al. 2003). Hemangioblast markers (Scl, VEGF receptor) and several early blood cell markers (GATA2, GATA1, Lmo2) are turned on in the BI primordium even before blood islands become morphologically distinct. As BI mature (**Figure 8*e–g***), GATA2, GATA1, and Scl are maintained strongly in the inner cells, which are thereby specified as hemocyte progenitors. Lmo2 is upregulated in the external (endothelial) cells, which form the capillary network surrounding the yolk sac. In mature yolk

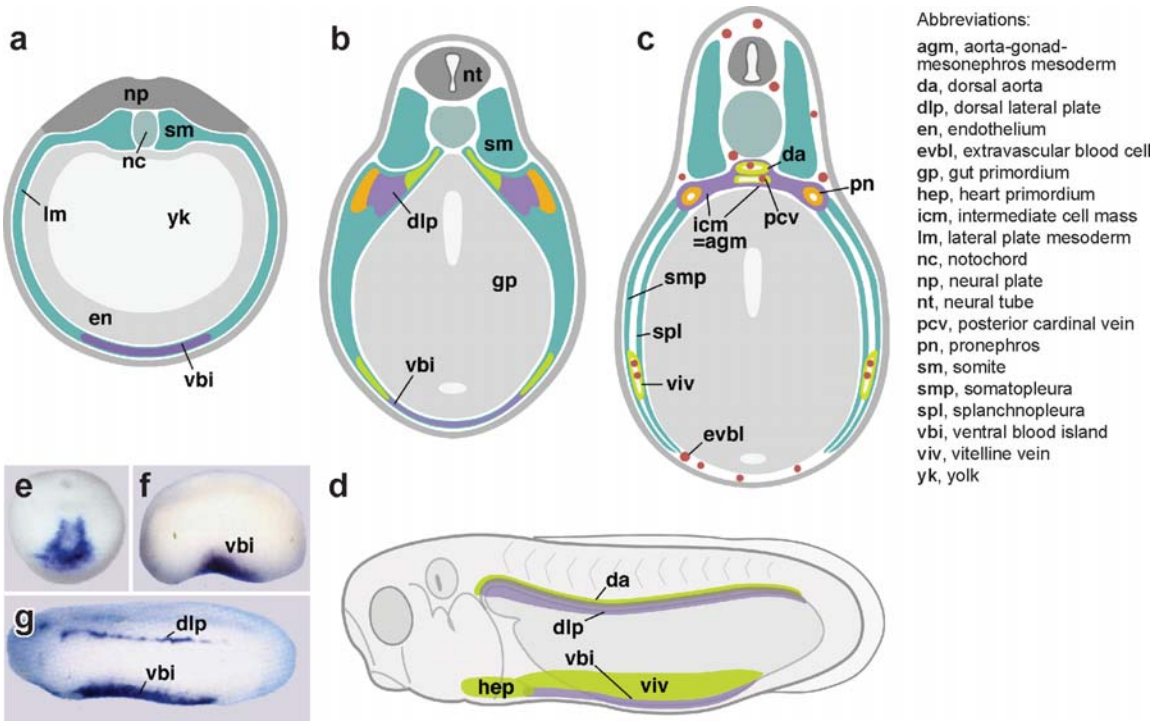


Figure 7

Early hematopoiesis in *Xenopus*. (*a–c*) Schematic cross sections of *Xenopus* embryo (*a*) at the end of gastrulation (stage 14, 10 h), (*b*) after neurulation (stage 26, 15 h), and (*c*) during the late stage of organogenesis (stage 36, 24 h). Two separate domains within the lateral plate mesoderm (lm) give rise to primitive and definitive blood cells. The ventral blood island (vbi) appears within the mid–ventral lateral plate and acts as the source of primitive hematopoiesis (*a*). The same cells also produce the endothelial cells of the vitelline veins (viv) (*c*). Slightly later than the appearance of the ventral blood island, cells of the dorsal lateral plate (dlp) express markers of hemangioblasts (*b*). The dorsal lateral plate migrates medially and forms blood vessels (da, dorsal aorta; pcv, posterior cardinal vein) and blood progenitors. It corresponds to the intermediate cell mass (icm) of zebrafish and the aorta-gonad-mesonephros mesoderm (agm) of amniotes. (*d*) Schematic lateral view of stage 26 *Xenopus* embryo showing primordium of the vascular system (*green*) (hep, heart primordium; da, dorsal aorta; viv, vitelline veins) and hematopoietic system (*violet*) (dlp, dorsal lateral plate; vbi, ventral blood island). (*e–g*) Expression of hemangioblast marker Scl in ventral blood island (vbi) and dorsal lateral plate (dlp) of (*e*) stage 14, (*f*) stage 20, and (*g*) stage 26 embryos. Panels *e–g* from Walmsley et al. 2002, with permission.

vessel these transcription factors are turned off and reappear in the intraembryonic (lateral plate) mesoderm at the stage at which definitive hemangioblasts are specified (Minko et al. 2003). In mouse embryos, as in chicken, blood islands forming both blood and endothelial cells can also be detected in the yolk sac. Recent experimental studies showed that the commitment of hemangioblasts takes place in the posterior primitive streak of the gastrulating embryo (**Figure 8*b*,*i***). As in chicken, these committed hemangioblasts express specific molecular markers, including scl, GATA1/2, and Flk1 (Dumont et al. 1995, Kallianpur et al. 1994, Silver & Palis 1997, Yamaguchi et al. 1993). In the yolk sac, angioblasts (expressing Flk1) and blood progenitors (marked by CD41) form adjacent yet nonoverlapping cell populations (Ferkowicz & Yoder 2005). Thus, rather than forming discrete "blood islands, blood progenitors lie together in a continuous zone, the 'blood band'" (**Figure 8*j–l***)

(Ferkowicz & Yoder 2005). This band borders a zone of vascular progenitors that form an endothelial plexus (**Figure 8*j–l***).

HSCs that start definitive hematopoiesis of birds and mammals can first be identified in the wall of the aorta, the gonadal mesoderm, and the mesonephros (AGM) (**Figure 8*m–q***) (Dieterlen-Lievre & Martin 1981, Ma et al. 2002, Medvinsky & Dzierzak 1996, Miles et al. 1997, Robin et al. 2003) as well as the yolk sac. It is thought that HSCs derived from both the AGM and the yolk sac settle, via the bloodstream, the hematopoietic tissues, first (and transiently) liver and spleen and then the bone marrow. It is not yet clear whether, in mouse and chicken, the same population of hemangioblasts that arose in the primitive streak and gave rise to primitive hematopoiesis also acts as the source for definitive hematopoiesis (as in zebrafish) or whether there exist two spatially separate cell populations in the early embryonic mesoderm (as in *Xenopus*).

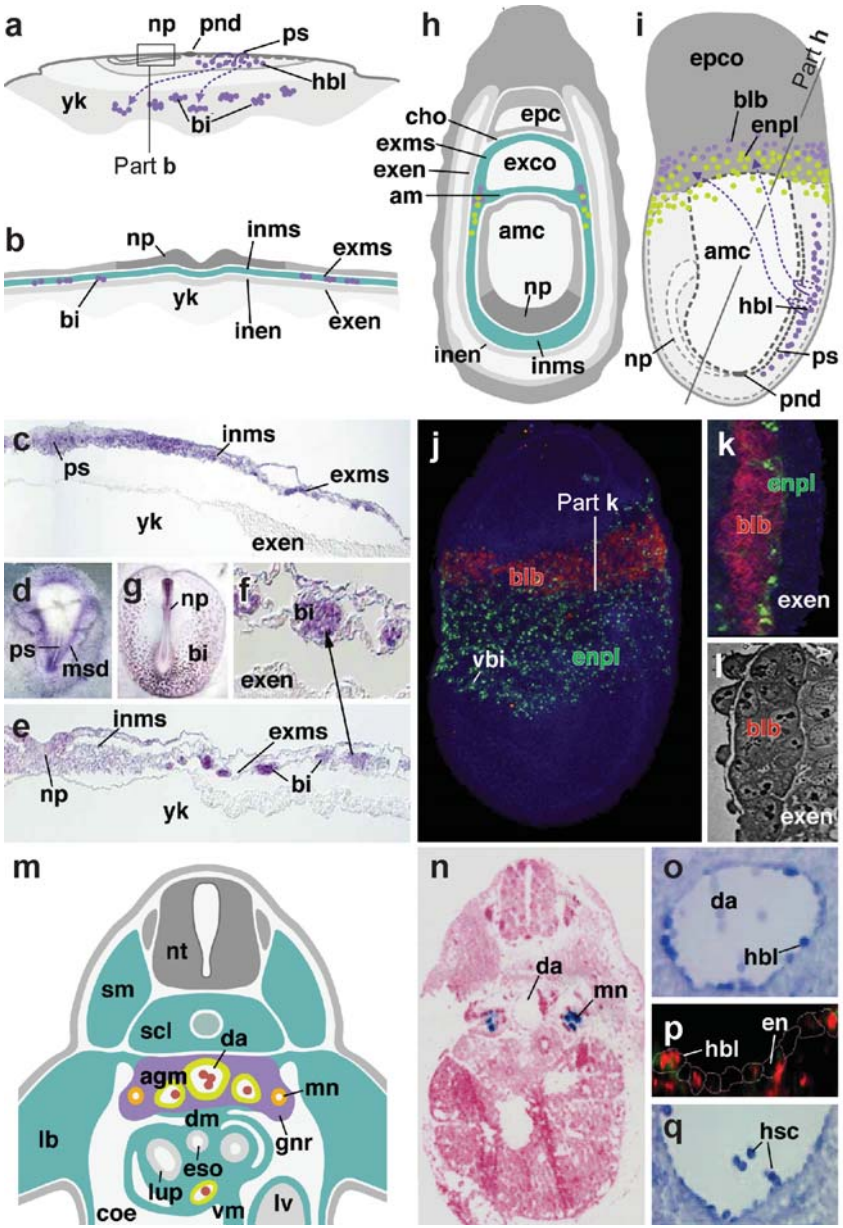
From this brief overview of early hematopoiesis in different vertebrate taxa, it appears that hemangioblasts are specified at an early stage within the lateral mesoderm. What is known about the signaling pathways that must become active to specify hemangioblasts and, in a second step, to separate the vascular (endothelial) lineage from the blood lineage? Hemangioblasts are induced from naive mesoderm by several signaling pathways that include BMP, FGF, VEGF, and Shh (**Figure 9*a***). The expression of BMPs in the lateral plate is required for upregulating determinants such as the GATA factors, Scl and Lmo2, and thereby specifies and maintains the fate of hemangioblasts. (Baron 2003, 2005; Crosier et al. 2002; Maeno 2003). In mutants of BMP2 or BMP4, many derivatives of the lateral plate, including the blood, vascular system, and visceral musculature, are missing or severely defective. Interestingly, the same phenotype occurs in *Drosophila* mutants lacking the BMP homolog, Dpp, which hints at the long evolutionary history behind the lateral mesoderm, its derivatives, and control by the BMP signaling pathway (see below).

FGF, along with BMP, is expressed in both lateral plate mesoderm and adjacent endoderm (Huber et al. 1998, Iraha et al. 2002). FGF is also one of the earliest signals that induces mesoderm prior to gastrulation. Mesoderm explants exposed to FGF form endothelial cells and blood cells. However, isolated mesoderm does not give rise to endothelial vessels with a lumen, and numerous studies have shown that externally derived signals are important to initiate vasculogenesis and hematopoiesis (Vokes & Krieg 2002). At the time of hemangioblast induction, VEGF and Shh enhance the intrinsic capacity of extraembryonic mesoderm or lateral mesoderm to produce both blood and vascular progenitors and, at the same time, direct the migration and differentiation of these cells (**Figure 9*a***) (Dyer et al. 2001, Eichmann et al. 1997, Gering & Patient 2005, Hiratsuka et al. 2005, Liang et al. 2001, Shalaby et al. 1995).

Little is known as yet about the signaling step that specifies the hemocyte progenitor from the bi- (oligo-?) potential hemangioblast. Definitive hemocyte progenitors emerge in the aortic endothelium, where they first reveal their fate upon upregulating GATA2, Scl, AML1, and Cbfa2 (Robert-Moreno et al. 2005). Thus, endothelial cells lining the embryonic aorta and other vessels are not terminally committed to the endothelial fate but rather carry the potential to become hemocytoblasts. It has recently been shown that Notch activation results in the expression of these determinants in the endothelial hemangioblasts, placing the Notch signaling pathway high up in the molecular network initiating hematopoiesis (**Figure 9*b***) (Burns et al. 2005, Hadland et al. 2004, Kumano et al. 2003, Robert-Moreno et al. 2005). Again, the same switch between vascular cells and blood progenitors is under the control of Notch signaling in *Drosophila* (Mandal et al. 2004) (see below).

Once they settle the bone marrow through the bloodstream, hemocytoblasts become

self-renewing HSCs that maintain the expression of factors such as GATA2, Scl, Lmo2, and AML1 (**Figure 9*c***); these proteins may keep HSCs in a proliferative, self-renewing state. Two of the well-studied signals that promote the HSC cell type within the stem cell niche are stem cell factor (SCF) and angiopoietin 1 (Ang1). SCF is expressed widely in the stroma and blood vessels of the bone marrow (Driessen et al. 2003). Ang 1 is expressed by the osteoblasts that surround the bone marrow. As a result, Ang1 reaches only the more peripheral hematopoietic cells that come into contact with the osteoblast wall, and these cells behave as self-renewing HSCs (Arai et al. 2004).



Abbreviations:
agm, aorta-gonad-mesonephros mesoderm
am, amniotic membrane
amc, amniotic cavity
bi, blood islands
blb, blood band
cho, chorionic membrane
coe, coelomic cavity
da, dorsal aorta
dm, dorsal mesentery
en, endothelium
enpl, endothelial plexus
epc, ectoplacental cavity
epco, ectoplacental cone
eso, esophagus
exco, extraembryonic coelom
exen, extraembryonic endoderm
exms, extraembryonic mesoderm
gnr, gonadal ridge
hbl, hemangioblast
hsc, hematopoietic stem cell
inen, intraembryonic endoderm
inms, intraembryonic mesoderm
lb, limb bud
lup, lung primordium
lv, liver primordium
mn, mesonephros
msd, mesoderm
np, neural plate
nt, neural tube
pnd, primitive node
ps, primitive streak
scl, sclerotome
sm, somite
vbi, ventral blood island
vm, ventral mesentery
yk, yolk

The Origin of Hemocyte Progenitors in *Drosophila*

Drosophila hemocyte progenitors (the equivalent of the hemocytoblasts discussed above) are born during two developmental phases from different populations of mesodermal cells (**Figure 10**). The lymph gland, described above as the hematopoietic organ producing hemocytes in the mature animal, is formed by hemocyte progenitors that arise in the lateral mesoderm of the trunk, a domain termed the cardiogenic mesoderm (**Figure 10*c*,*d***) (Crozatier et al. 2004, Mandal et al. 2004, Rugendorff et al. 1994). The cardiogenic mesoderm has been likened to the vertebrate AGM mesenchyme because both structures give rise not only to blood but also to endothelial cells and nephrocytes. In the late embryo, the lymph gland is formed by a paired cluster of approximately 20 cells flanking the anterior part of the dorsal blood vessel (aorta) (**Figure 10*e***). The cells aligned on either side of the dorsal vessel posterior to the lymph gland are the pericardial nephrocytes, which function as excretory cells. Progenitors of the lymph gland can be recognized through their expression of the GATA1–3 homolog Srp (Evans et al. 2003, Sorrentino et al. 2005) along with other transcription factors, among them Odd skipped (a zinc finger protein with no known function in vertebrate hematopoiesis) (Mandal et al. 2004) and Collier (the homolog of EBF)

←

Figure 8

Early hematopoiesis in chicken and mouse. (*a*) Schematic lateral view of chicken embryo at stage 5 (20 h; onset of somite formation; anterior to the left). Primitive node (npd) and primitive streak (ps) behind it mark the domain of ingressing mesoderm. Presumptive hemangioblasts (hbl) expressing blood and blood vessel markers (*violet*) appear in nascent mesoderm and migrate laterally and anteriorly (*arrows*), coalescing into discrete blood islands (bi). (*b*) Schematic cross section of stage 5 chicken embryo showing spatial relationship of neural plate (np) ectoderm, intra- and extraembryonic mesoderm (inms, exms), and intra/extraembryonic endoderm (inen, exen). Blood islands (bi) appear in extraembryonic mesoderm of yolk sac. (*c*) Cross section of stage 6 embryo, showing widespread expression of GATA2 in intraembryonic and extraembryonic mesoderm. (*d*) Dorsal view of stage 5 chicken embryo showing GATA2 in mesoderm (msd) that is ingressing through primitive streak (ps). (*e*) Cross section of stage 10 chicken embryo (30 h) with GATA2 expressed in discrete blood islands (bi) scattered throughout extraembryonic mesoderm. (*f*) High magnification of blood island (bi) marked through GATA2 expression. (*g*) Dorsal view of stage 10 chicken embryo showing expression of the hemangioblast marker Scl in blood islands (bi) (*d–g* from Minko et al. 2003, with permission). (*h*,*i*) Schematic representations (*h*: cross section; *i*: lateral view; *diagonal line* in *i* indicates plane of section represented in *h*) of 7.5-day mouse embryo during late gastrulation. Germ layers line the amniotic cavity (amc), with ectoderm (np, neural plate) facing inward. Mesoderm ingressing through primitive streak (ps) spreads out to form intraembryonic and extraembryonic mesoderm (inms, exms). Hemangioblasts (hbl) (*violet*) are specified in primitive streak and, after migrating, form blood band (blb) and endothelial plexus (enpl) around the border between extraembryonic and embryonic mesoderm. (*j*) Wholemount of stage 7.5 mouse embryo showing expression of endothelial marker Flk1 (*green*) and hematopoietic stem cell marker CD41 (*red*). (*k*,*l*) Section of extraembryonic wall containing blood band (blb) (labeled by CD41, *red*, in *k*) and endothelial plexus (enpl) (labeled by Flk1 expression, *green*, in *k*; see *white line* in panel *j* for orientation of section shown in *k* and *l*). Note adjacent yet not overlapping location of blood and endothelial progenitors (*j–l* from Ferkowicz & Yoder 2005, with permission). (*m*) Schematic cross section of 11.5-day mouse embryo. The roof of coelomic cavity (coe) houses major blood vessels (da, dorsal aorta), the excretory system (mn, mesonephros), and gonad primordium (gnr, gonadal ridge). All these structures are surrounded by and include mesoderm (agm) that contains hemangioblasts. (*n*) Cross section of stage 11.5 mouse embryo showing expression of HSC marker Sca1 in mesonephros (mn) (from Miles et al. 1997, with permission). (*o–q*) Cross sections of aorta of 9.5-day mouse embryo. (*o*) Expression of AML1 (*blue*) in hemangioblasts (hbl) located in the aortic endothelial wall (en). (*p*) Coexpression of GATA2 (*red*) and Notch (*green*) in hemangioblasts (hbl). (*q*) Expression of Hes 1 (downstream of activated Notch; *blue*) in hematopoietic clusters (hsc) budding from aortic wall. *o–q* from Robert-Moreno et al. 2005, with permission.

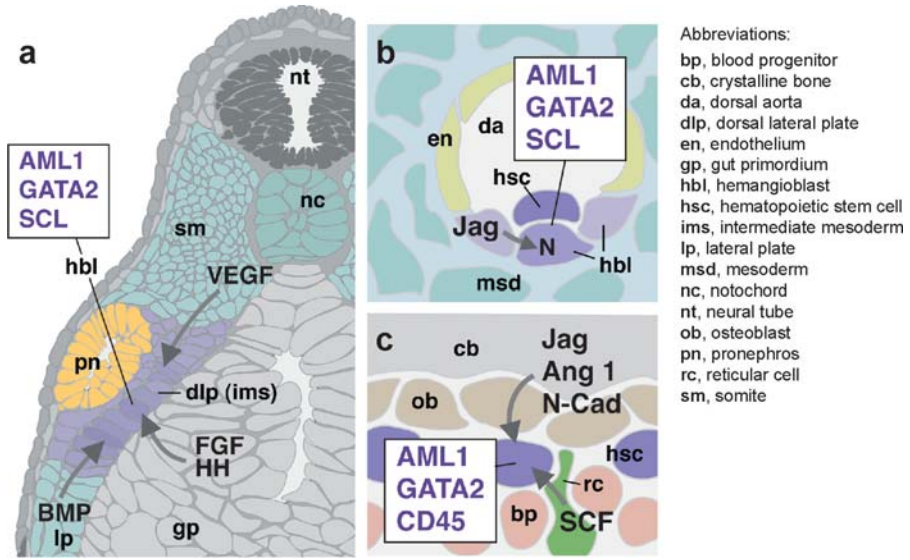


Figure 9

Signaling pathways involved in specification of hemangioblasts in vertebrate embryo. (*a*) Schematic cross section of postneurula vertebrate embryo (*Xenopus*). Mesodermal domain in between lateral plate (lp) and somitic mesoderm (sm)—referred to as the intermediate mesoderm (ims), dorsal lateral plate (dlp; in *Xenopus*), or intermediate cell mass (in zebrafish)—reacts to BMP, FGF, HH, and VEGF signals derived from neighboring tissues, including endodermal gut primordium (gp), lateral plate (lp), somitic mesoderm (sm), and notochord (nc). These signals trigger the expression of determinants of hematopoietic fate, including GATA2, AML1, and Scl. As a result, cells become hemangioblasts, which migrate dorsally to become part of the dorsal aorta and other mesodermal structures, such as the excretory system and gonadal primordium. (*b*) Schematic cross section of dorsal aorta (da), which contains hemangioblasts (hbl). Hemangioblasts can give rise to endothelial cells (en) and hematopoietic stem cells (HSCs) (hsc); the latter fate is triggered upon activation of the Notch (N) pathway by the ligand Jagged (Jag). (*c*) Schematic section of the HSC stem cell niche in the bone marrow. HSCs receive signals from neighboring osteoblasts (ob) (Jag, Angiopoietin 1, N-cadherin) and reticular cells (rc) (SCF, stem cell factor) that maintain the status of HSCs as self-renewing.

(Crozatier et al. 2004). These early markers reveal that the lymph gland progenitors form three metameric clusters in the cardiogenic mesoderm of the thoracic segments. Clonal analysis demonstrated that, similar to what long has been observed in vertebrates, hemocyte progenitors are closely related to the cells forming the dorsal vessel (cardioblasts). Two-cell clones containing one hemocyte progenitor and one cardioblast were recovered, supporting the notion that the *Drosophila* lateral mesoderm houses bipotential hemangioblasts (Mandal et al. 2004).

Whereas hemocytes produced in the lymph gland differentiate in the larva and function from the late larval into the adult phase (Lanot et al. 2001), the embryo and early larva are populated by hemocytes that are born during an earlier phase, shortly after gastrulation, from the mesoderm of the head (Evans et al. 2003, Schulz & Fossett 2005, Tepass et al. 1994; **Figure 10*a*,*b***). As these primary hemocytes progress through the prohemocyte stage, they quickly spread out throughout the embryo, and most of them differentiate into phagocytosing plasmatocytes. The primary function of the primary hemocytes is to remove apoptotic cells that amass during normal embryonic development; the hemocytes are also involved in producing the ECM (basement membranes) that surrounds all tissues. It is tempting to compare

the early phase of hemocyte formation that occurs in the head mesoderm of *Drosophila*, and that produces the blood cells of the embryo and larva, with primitive hematopoiesis in vertebrates. Similarly, late hematopoiesis within the fly lymph gland could be likened to definitive hematopoiesis in vertebrates. It remains to be established with molecular markers whether this comparison has any merit.

Transcription factors and signaling pathways acting in the vertebrates during the specification of hemocyte progenitors appear to be conserved to a high degree in *Drosophila*. FGF, BMP, and Wnt/Wg signaling are sequentially involved to specify the cardiogenic mesoderm (**Figure 10*b′*,*c′***). Loss of function of the proteins Heartless (Htl) (*Drosophila* homolog of one FGF receptor) (Beiman et al. 1996), Decapentaplegic (Dpp) (homolog of BMP2/4) (Frasch 1995, Staehling-Hampton et al. 1994), and Wingless (Wg) (homolog of Wnt) (Lockwood & Bodmer 2002, Wu et al. 1995) results in the absence of all derivatives of the cardiogenic mesoderm, including secondary hemocytes, cardioblasts, and pericardial nephrocytes (Mandal et al. 2004). FGF and BMP, as well as Hedgehog, signaling also act in an as-yet-ill-defined manner during later stages of hematopoiesis in *Drosophila* (Johnson et al. 2003; V. Hartenstein, unpublished data). VEGF, one of the essential signals controlling the specification of hemangioblasts as well as vascular differentiaton in vertebrates, also appears to act at a late step of hematopoiesis in *Drosophila*. Loss-of-function studies of PVR, the *Drosophila* homolog of VGFR and the platelet-derived growth factor (PDGF) receptor, indicate that both migration and maintenance of hemocytes require this signaling pathway (Bruckner et al. 2004, Cho et al. 2002, Heino et al. 2001, Munier et al. 2002).

A pivotal step in hemocyte progenitor determination is the expression of the GATA factor Srp (homolog of GATA1–3) (Mandal et al. 2004, Rehorn et al. 1996). As discussed above, this factor also acts as one of the earliest determinants in vertebrate blood formation. The maternal systems turn on Srp in the early head mesoderm (**Figure 10*a***); in the cardiogenic mesoderm of the trunk, input from Htl, Dpp, and Wg signaling pathways is required (**Figure 10*b′*,*c′***). Homologs of other early determinants of vertebrate hemocytoblasts act during later stages in *Drosophila* hematopoiesis (e.g., the AML1 homolog Lozenge (Lz) (Lebestky et al. 2000) (see below) or other, unrelated pathways [e.g., the *Drosophila* Scl homolog (Varterasian et al. 1993) or the C/EBP homolog (Montell et al. 1992)]. In turn, the vertebrate homolog of the early-acting *Drosophila* blood determinant Gcm plays a role in numerous later developmental pathways in vertebrates, including those involved in the differentiation of the placenta, thymus, and kidney (Hashemolhosseini & Wegner 2004). Likewise, the vertebrate *odd*-related gene is involved in skeleton specification and patterning (Lan et al. 2004, So & Danielian 1999) and has not yet been confirmed as a factor acting during early hematopoiesis.

In vertebrates, the Notch signaling pathway plays a pivotal role in promoting multipotent HSCs (Robert-Moreno et al. 2005); in addition, Notch acts during the lineage specification of T lymphocytes (for a recent review, see Radtke et al. 2005). Notch also functions at multiple steps in *Drosophila* hematopoiesis. At an early stage, Notch represents the switch in the cardiogenic mesoderm that allows for hemocyte formation. Thus, if Notch function is absent from the cardiogenic mesoderm, all its cells turn into vascular cells (**Figure 10*d′***) (Hartenstein et al. 1992, Mandal et al. 2004). Secondly, Notch is required for the fate of crystal cells, as is discussed in more detail in the next section.

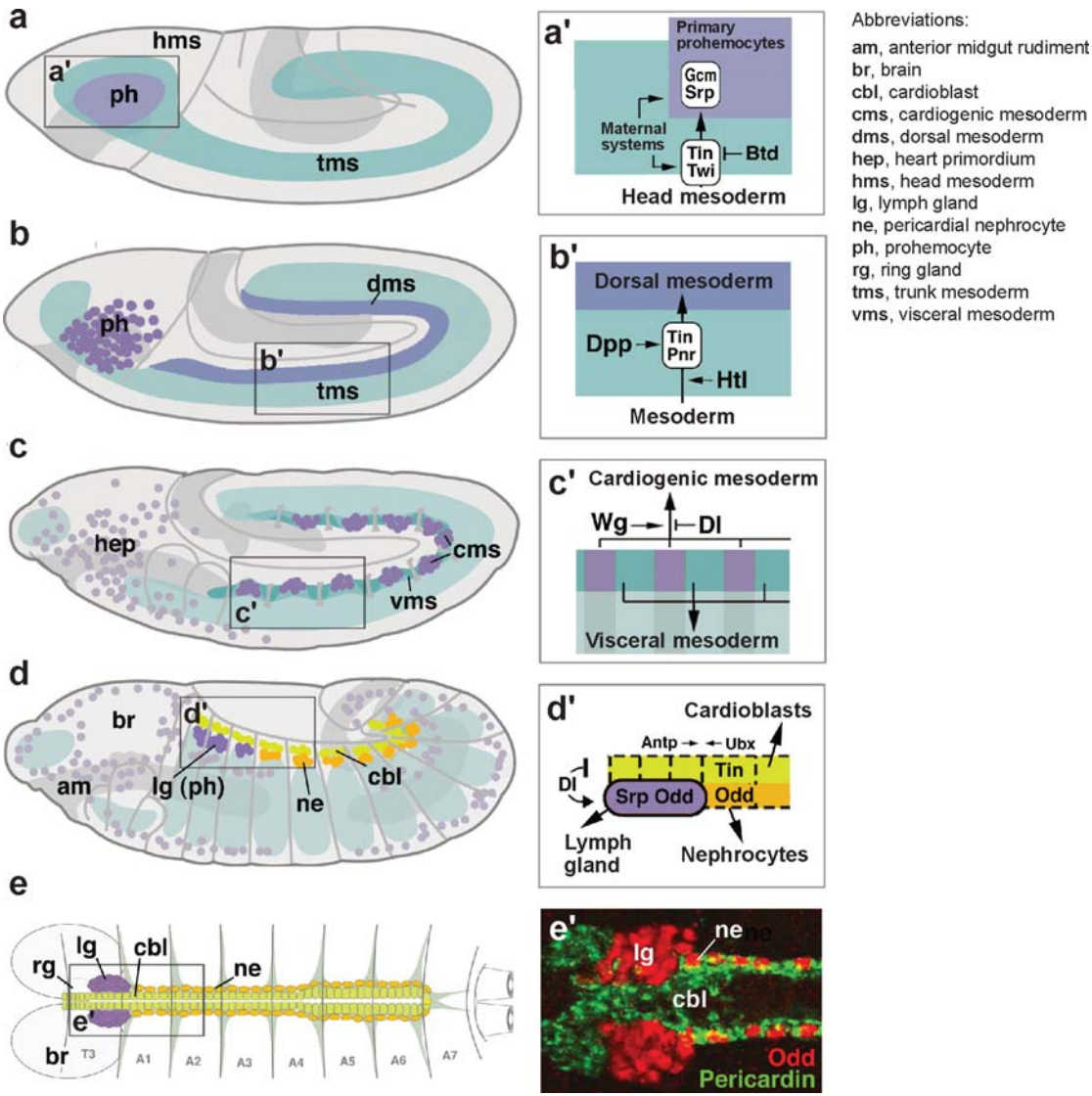
GENERATION OF HEMOCYTE DIVERSITY

Hemocytes of highly derived animals, both vertebrate and invertebrate, fall into multiple classes with specialized functions. It is reasonable to assume that this diversity of

hemocytes took its origin from a single ancestral cell type that may have played a role in phagocytosis and/or digestive functions. An attempt was made in the first part of this review to draw parallels between the major structurally defined classes of hemocytes encountered in different animal phyla. For a more detailed and well-founded comparison, molecular markers, as well as thorough developmental analyses of hemocyte origins and lineage diversification, would be needed. Data of this sort so far exist only for vertebrates and *Drosophila*. In the last section of this review, some of the main molecular principles that guide the diversification of blood cells in vertebrates and *Drosophila* are discussed to highlight the similarities of and differences between these phylogenetically distant taxa.

Blood Cell Lineages in Vertebrates

When HSCs leave the stem cell niche, they enter the phase of dedicated progenitor cells (also called colony-forming units,



Abbreviations:
am, anterior midgut rudiment
br, brain
cbl, cardioblast
cms, cardiogenic mesoderm
dms, dorsal mesoderm
hep, heart primordium
hms, head mesoderm
lg, lymph gland
ne, pericardial nephrocyte
ph, prohemocyte
rg, ring gland
tms, trunk mesoderm
vms, visceral mesoderm

or CFUs). This means that (*a*) cells lose their totipotency (that is, they become committed to one or a few blood cell fates) and (*b*) they increase proliferation. First, two progenitor cell types, lymphoid multipotential cells and myeloid multipotential cells, are formed. The former are the progenitors of lymphocytes, whereas the latter produce all other blood cells (Ling & Dzierzak 2002, Orkin 2000). Myeloid multipotential cells split into progenitors of erythrocytes, megakaryocytes/thrombocytes, neutrophilic granulocytes/monocytes, eosinophilic granulocytes, and basophilic granulocytes. The stage of rapidly dividing progenitors is followed by the precursor stage, during which proliferation slows down and differentiation of blood cells sets in. At this precursor stage, the blood cells are termed -blasts, such as monoblasts, myeloblasts, and erythroblasts.

Some of the transitions in the expression pattern of transcriptional regulators that control different blood cell fates have been investigated in great detail. Of particular interest in this comparative context is the granulocyte/monocyte (GM) progenitor—which gives rise to two lineages, the neutrophilic granulocytes ("G") and monocytes/macrophages ("M")—and the erythrocyte (E) progenitor. Characteristic of GM progenitors is the expression of the transcription factor PU.1, which activates target genes such as that of the granulocyte/macrophage-colony-stimulating factor (GM-CSF) receptor (**Figure 11*a***) (Gangenahalli et al. 2005). By doing so, the GM progenitor acquires the capability of rapid proliferation. Studies of the gene structure of PU.1 have also answered the question of why this gene is not active in HSCs residing in the stem cell niche. Thus, the PU.1 protein possesses binding sites for GATA2 as well as GATA1 (Nerlov et al. 2000, Zhang et al. 1999). These two act as repressors of PU.1. Only after the HSC loses GATA2 can PU.1 be expressed and in turn switch on the GM-CSF receptor. By contrast, the maintenance of high levels of GATA2 through, for example, forced Notch activation inhibits the progression from HSC to specific progenitor and thereby blocks hematopoiesis (Kumano et al. 2001). One has to envisage transcription factors like GATA2 (and many others) as

Figure 10

Early hematopoiesis in *Drosophila*. (*a–d*) Schematic lateral views of *Drosophila* embryos at stages 7 (*a*), 10 (*b*), 11 (*c*) and 12 (*d*). Panels *a′* to *d′* illustrate known essential molecular mechanisms acting at the corresponding stages. (*a*,*a′*) Early wave of hematopoiesis in the head mesoderm (hms). Maternal dorsoventral system specifies mesoderm by activating determinants such as Twist (Twi) and Tinman (Tin). For specification of prohemocytes (ph) to occur, Tin is downregulated by head gap gene Buttonhead (Btd; SP1 transcription factor), which in turn is activated by the anterior maternal system. The anterior system is also required to regulate prohemocyte determinants Srp (GATA1–3 homolog) and Gcm positively. (*b*,*b′*) Prohemocytes (ph) resulting from early wave of hematopoiesis in head mesoderm begin spreading out throughout the embryo. The trunk mesoderm (tms) stretches dorsally, requiring activity of the Heartless (Htl; FGF homolog) signal. Dorsal mesoderm (dms) receives Dpp signal (BMP2/4 homolog) from dorsal ectoderm, which triggers expression of Srp and maintains expression of Tin. (*c*,*c′*) Initiation of the second wave of hematopoiesis in dorsal trunk mesoderm. Among other signals, Wg (Wnt 1) and Delta (Dl) are required to specify within the dorsal mesoderm segmental clusters of cells with cardiogenic fate (cms). The remainder of the dorsal mesoderm develops as visceral mesoderm (vms). (*d*,*d′*) The cardiogenic mesoderm splits up into three major lineages: cardioblasts (cbl), pericardial nephrocytes (ne), and hemocyte progenitors [prohemocytes (ph)] [located in lymph gland (lg)]. Notch/Delta (N/Dl) signaling is positively required for nephrocytes and lymph gland. Srp becomes restricted to the lymph gland, and Tin to cardioblasts; Odd (Odd skipped) marks lymph gland and nephrocytes. Input from the Hox complex (Antp is positively required; Ubx is negatively required) specifies lymph gland in the three thoracic segments, whereas abdominal segments produce nephrocytes. (*e*,*e′*) Schematic diagram (*e*) and photograph (*e′*) of dorsal vessel and lymph gland of late *Drosophila* embryo, dorsal view. A1–A7, abdominal segments 1–7.

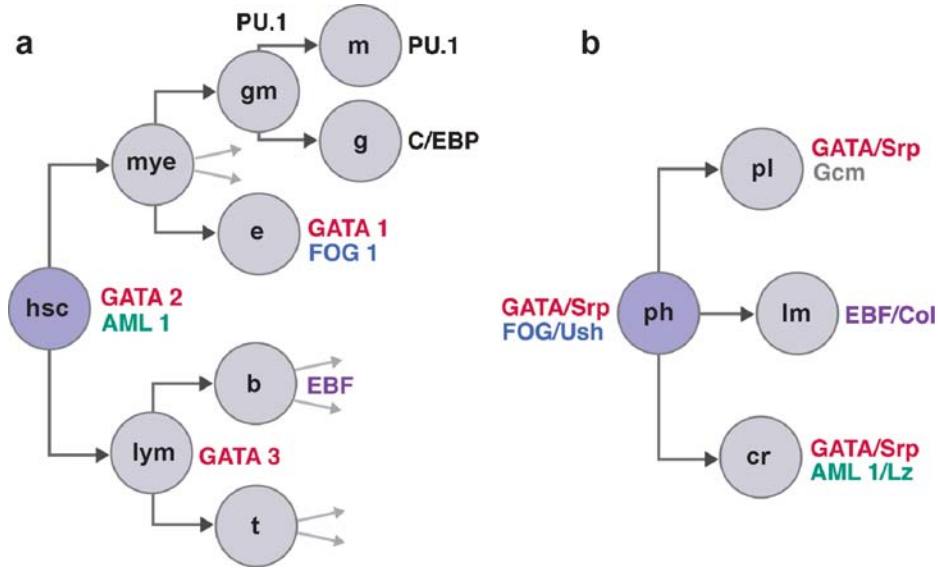


Figure 11

Diversification of blood lineages and determinates of blood cell fates in (*a*) vertebrates and (*b*) *Drosophila*. Conserved determinants of blood cell fates (GATA factors, AML1/Lz, FOG/Ush, EBF/Col) are in various text colors. Some of the essential hematopoietic determinants that show no conservation between vertebrates and *Drosophila* (C/EBP, PU.1, Gcm) are in gray. Abbreviations of blood cell lineages: b, B lymphocytes; cr, crystal cells; e, erythrocytes; g, neutrophilic granulocytes; gm, progenitors of neutrophilic granulocytes and monocytes; hsc, hematopoietic stem cell; lm, lamellocytes; lym, lymphoid progenitor; mye, myeloid progenitor; m, monocytes; ph, prohemocytes; pl, plasmatocytes; t, T lymphocytes.

molecules that transiently turn on in immature blood cell progenitors and "push" these cells through a certain developmental phase but that have to be switched off for terminal differentiation to occur.

GM progenitors produce both monocytes and granulocytes. The switch between the two is caused by the induction of the C/EBP transcription factor in cells that are embarking on the path toward becoming granulocytes (Lekstrom-Himes 2001, Friedman 2002). This factor represses PU.1 function and activates the expression of yet another cytokine receptor, the G (granulocyte)-CSF and IL-6, two signals that promote granulocyte growth (**Figure 11*a***) (Zhang et al. 1998). Cells that do not turn on C/EBP maintain high expression of PU.1, which then activates the receptor for M (monocyte)-CSF (Friedman 2002, Gangenahalli et al. 2005).

In the erythroid progenitor, GATA1 expression replaces GATA2. Another transcription factor, FOG 1, is a binding partner of GATA1 (Cantor & Orkin 2002). GATA1 also blocks PU.1, so the erythroid progenitor does not switch on the receptor for GM-CSF. Instead, GATA1 itself turns on the receptor for erythropoietin, the cytokine that stimulates erythrocyte proliferation. GATA1 also activates structural genes required in erythrocytes, such as globin.

Lymphocytes, the mediators of the adaptive immune system, are specified from the multipotential lymphoid progenitors in a similar way as for the myeloid lineages (discussed above). Lymphocyte lineages leave the bone marrow and mature in lymphoid organs, including the thymus, lymph nodes, and spleen; these provide specialized microenvironments for the expression of factors that move

lymphocytes along their distinctive pathways of differentiation. One factor, required for early B lymphocyte development, is early B cell factor (EBF) (Smith et al. 2005); the lack of EBF results in an early developmental blockade, including the lack of functional B cells and of immunoglobulin production.

A large number of cytokines that turn on and off transcriptional regulators of blood cell fate at the appropriate times have been identified. Based on their function, one can distinguish stem cell factors that promote maintenance of HSCs (such as SCF) (Nishikawa et al. 2001), multilineage factors that act on several lineages (for example GM-CSF or IL-3) (Barreda et al. 2004), and lineage-specific factors (such as G-CSF for neutrophilic granulocytes or EPO for erythrocytes) (Barreda et al. 2004, Richmond et al. 2005). The receptors for most of these cytokines fall into two different classes. One class is that of the receptor kinases, which includes the receptors for VEGF, FGF, EGF, and PDGF. The receptor for M-CSF is very similar to those for PDGF and VEGF. Evolutionarily, all three receptor genes probably arose by duplication in the early vertebrate lineage (Suga et al. 1999). Thus, invertebrates like *Drosophila* have a single receptor, PVR, involved in blood/vascular cell development (see below). Correspondingly, they have a relatively simple blood vascular system with few endothelial cells and only three types of hemocytes. In vertebrates, the diversity of hemocytes has increased considerably. As gene duplications amassed during evolution, they could be utilized to generate a wider spectrum of signals/receptors with different functions.

Drosophila

The multipotential blood progenitors of *Drosophila* differentiate into three morphologically and functionally distinct cell classes (Evans et al. 2003, Lanot et al. 2001, Meister & Lagueux 2003, Schulz & Fossett 2005). The largest class is composed of hyaline plasmatocytes; the two other, smaller blood cell populations are composed of crystal cells and lamellocytes. Both of the latter cell types are crucially involved in the innate immune response of *Drosophila*. Thus, whereas plasmatocytes are the generic phagocytes, involved in removing cell debris that has resulted from apoptosis as well as in wound repair, crystal cells and lamellocytes act in encapsulating foreign invaders that have made their way into the fly larva. The most common infections threatening the larva are those by fungi and parasitic wasps, which deposit their eggs into its body cavity. Such foreign intruders are rapidly surrounded by crystal cells and plasmatocytes, both of which form a tough, pigmented capsule that most often kills the intruding organism.

In regard to their function, lamellocytes and crystal cells may comprise a highly specialized type of hemocytes that has no counterpart in the vertebrate set of hemocytes. Plasmatocytes, on the other hand, are comparable with the monocyte/granulocyte lineage, which, in vertebrates, is responsible for phagocytosis and multiple steps of the innate immune response. Molecular analysis of *Drosophila* blood cells is compatible with this conclusion: A homolog of GATA1/2/3 is the *Drosophila* Serpent (Srp) (Rehorn et al. 1996, Sorrentino et al. 2005). This is the earliest marker of hemocyte precursors in the embryo and larva and is essential for all hemocytes (**Figure 11*b***). One could conclude that in the Bilaterian (fly-vertebrate) ancestor, one protein like GATA1–3/Srp existed; this situation was maintained in *Drosophila*, in which little diversification of blood cell types occurred, but became more complex in vertebrates. Three proteins, GATA1–3, evolved; these took on specific roles in hemocyte diversification (i.e., GATA2 for HSCs, GATA1 for the erythroid lineage, and GATA3 for the lymphoid lineage).

A cofactor crucial for GATA function in vertebrates, FOG1/2, is also conserved in *Drosophila*. It is represented by the protein U-shaped (Ush), which acts both in early

hematopoiesis in the head mesoderm and later in specification of the cardiogenic mesoderm/lymph gland (**Figure 11*b***) (Evans et al. 2003, Fossett et al. 2001). As in vertebrates, both GATA factors and FOG are under the control of BMP signaling. Dpp, similar to its vertebrate counterpart BMP2/4, acts upon the cardiogenic mesoderm and has the role (among others) of turning on Srp and Ush transcription (Mandal et al. 2004).

A transcription factor acting as one of the first and most general specifiers of hematopoiesis in vertebrates, AML1, has a *Drosophila* counterpart with a much more restricted role. The *Drosophila* AML1 homolog is Lozenge (Lz), which is required only for the formation of crystal cells (Lebestky et al. 2000). We are faced with two possibilities as to the evolutionary scenario that defined the role of AML1/Lz. First, this gene may have started out with a restricted (and late) function in the Bilaterian ancestor, which is possible because other factors with early/general function existed in the form of GATA factors (e.g., GATA2 in HSCs). In this case, this original condition was preserved in *Drosophila* and then changed in vertebrates, in which AML1 took on an early role alongside GATA2. Alternatively, AML1 may have started out as an early factor, which is still the case in vertebrates, and gradually became more restricted in function in the line of animals leading up to *Drosophila*.

The *Drosophila* homolog of EBF, Collier, has recently been shown to play a role in the formation of lamellocytes (Crozatier et al. 2004). This transcription factor is expressed in a small subset of cells within the lymph gland, termed the posterior signaling center (PSC). In *collier* mutations, the PSC is not maintained during larval stages, and lamellocytes do not form.

Lineage specification and maturation of *Drosophila* hemocytes require input from several signaling pathways. Activation of the Notch pathway is required not only for the early specification of prohemocytes (see above) but also for the induction of crystal cells (Lebestky et al. 2003). PVR (the receptor homologous to the vertebrate VEGF and PDGF receptors) and JAK/STAT signaling (the same pathway that transmits cytokine signals in vertebrate hematopoiesis) are involved in the specification and differentiation of plasmatocytes (Agaisse & Perrimon 2001, Bruckner et al. 2004, Cho et al. 2002, Evans et al. 2003, Heino et al. 2001).

In conclusion, the comparison of invertebrate hemocytes with vertebrate hemocytes is still difficult. The diversity of hemocytes within a given species is much less in invertebrates than in vertebrates. For example, lymphocytes, responsible for the adaptive immune response in vertebrates, are altogether lacking in invertebrates, which do not have this response. But what can be said about the remaining hemocytes found among the invertebrates? It is reasonable to liken the insect prohemocyte with early-stage cells in vertebrate hematopoiesis, such as the multipotential myeloid progenitor or even the HSC. Furthermore, regarding function and gene expression, the plasmatocyte (or hyaline hemocyte), which is the most numerous hemocyte in *Drosophila*, is the most similar to the vertebrate monocyte/histiocyte. Molecular markers—in the form of signals, receptors, or transcription factors that are characteristic for individual hemocyte types in vertebrates—may tell us more about relationships among hemocytes in the animal kingdom. The identification of such markers and their expression in hemocytes of invertebrate animals besides *Drosophila* promise to become a productive venue providing insight into blood cell evolution.

APPENDIX OF ABBREVIATIONS (WITH FUNCTIONS)

AGM, aorta-gonad-mesonephros; ALM, anterior lateral mesoderm; AML1, acute myeloic leukemia 1 protein (transcription factor); Ang1, angiopoietin1 (signal); bHLH, basic helix-loop-helix protein (transcription factor family); BI, blood islands; BMP,

bone morphogenetic protein (signal protein family); BrdU, bromo-deoxy-uridine; C/EBP, CCAAT/enhancer binding protein (transcription factor); Cbfa2, core binding factor alpha 2 (transcription factor); CFU, colony-forming unit; Col, Collier (Drosophila EBF homolog); Dl, Delta (signal protein); Dpp, Decapentaplegic (Drosophila BMP2/4 homolog); EBF, early B cell factor (transcription factor); ECM, extracellular matrix; EGF, epidermal growth factor (signal protein family); FGF, fibroblast growth factor (signal protein family); Flk1, fetal liver kinase 1 (signal receptor); FOG, friend of GATA (transcription factor family); GATA, DNA sequence motif (transcription factors binding to GATA); Gcm, glial cells missing (transcription factor); G-CSF, granulocyte-colony-stimulating factor (signal protein); GM-CSF: granulocyte/monocyte-colony-stimulating factor (signal protein); HSC, hematopoietic stem cell; Htl, Heartless (Drosophila FGF receptor); ICM, intermediate cell mass; IL-6, Interleukin-6 (signal protein); JAK, Janus kinase (signal transducer); Lmo2, LIM domain only 2 (transcription factor); Lz, Lozenge (Drosophila AML1 homolog); M-CSF, monocyte-colony-stimulating factor (signal protein); MHC, major histocompatibility complex protein (membrane protein); N, Notch (signal receptor); Pax, Paired-homeodomain protein (transcription factor family); PDGF, platelet-derived growth factor (signal protein); PLM, posterior lateral mesoderm; PSC, posterior signaling center; PU.1, purine-rich 1 (transcription factor); PVR, PDGF/VEGF receptor (signal receptor); SCF, stem cell factor (transcription factor); Scl, stem cell leukemia factor (transcription factor); Shh, Sonic hedgehog (signal protein); Srp, Serpent (Drosophila GATA1–3 homolog); Ush, U-shaped (Drosophila FOG homolog); VEGF, vascular endothelial growth factor (signal protein); Wg, wingless (Drosophila Wnt1 homolog); Wnt, (signal protein family)

ACKNOWLEDGMENT

This work was supported in part by NIH Grant RO1 HL67395 to Drs. Utpal Banerjee and V.H.

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Annual Review of Cell and Developmental Biology

Volume 22, 2006

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