1 Comparative aspects and possible phylogenetic affinities of vertebrate and invertebrate blood cells

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Introduction

All vertebrates possess blood cells responsible for respiratory, immune and haemostatic processes but, of these functions, only cellular and humoral immunity are mediated by complex interactions between subpopulations of sessile and freely circulating cells. Erythrocytes transport oxygen, thrombocytes or platelets initiate haemostasis while a heterogeneous array of T- and B-lymphocytes, macrophages, monocytes, basophils, mast cells, eosinophils and neutrophils are components of the vertebrate immune system. This system is highly evolved and extremely sophisticated so that most vertebrates respond to antigenic stimulation utilizing finely discriminative humoral and cellular processes involving the production of specific antibodies, lymphokines and memory cells. Even the agnathans, at the base of the vertebrate phylogenetic tree, synthesize antibody, exhibit allogeneic recognition, possess lymphocyte heterogeneity and plasma cells. The question thus arises as to the origin of vertebrate blood cells responsible for these immune and other reactions.

In this brief consideration of vertebrate blood cell origins, the problems of such a study are indicated, then an outline of the possible steps or factors involved in the phylogeny of the immune system is given, and, finally, comparisons are made between vertebrate and invertebrate blood cell types. Most of which is presented owes much to original concepts published by Burnet (1968, 1971), Marchalonis & Cone (1973), Manning (1975, 1979, 1980), Cooper (1976a,b, 1977, 1982), Marchalonis (1977), Hildemann *et al.* (1981), and by others, detailed below, to whom the reader is referred.

PROBLEMS IN TRACING THE ORIGINS AND EVOLUTION OF VERTEBRATE BLOOD CELLS

Unfortunately, since the ancestors of vertebrates are now extinct, there has been considerable controversy concerning their phylogenetic origins. It is often stated that echinoderm- or tunicate-like animals gave rise to the vertebrates, (Fig. 1) by way of a filter feeding larval form (Garstang 1928; Berrill 1955; Romer 1967), although the adult stage of these animals bears little resemblance to vertebrates. The idea of a prototunicate larva as the vertebrate ancestor has, however, more recently been challenged by Jollie (1973) since it seems likely to him that a filter feeder would lead to a

sessile or inactive life rather than an active protovertebrate with highly developed sensory, neural and locomotor systems. Thus, attempts to trace the origin of vertebrate blood cells within the invertebrates will at best be speculative and based on the assumption that comparisons with living species are valid since the enigmatic ancestral forms are no longer available.

Another problem emanating from the uncertainty of vertebrate ancestry is associated with the identification of truly homologous cells and structures (Cooper 1976a, 1977, 1982; Warr & Marchalonis 1978). By definition, homologous blood cells would have the same phylogenetic origins whereas analogous cells may resemble each other structurally or functionally but would not share a common ancestor. As we shall detail below, the coelomocytes of some annelids may resemble vertebrate lymphocytes in their response to certain mitogens and functions during graft rejection (see refs in Cooper 1981, 1982) but the cells involved are probably analogous due to the relatively distant phylogenetic positions of the annelids and the vertebrates. The annelids, together with the flatworms, molluscs and arthropods, are protostomes (Fig. 1) and characterised during development by determinate cleavage and a blastopore which divides to form both the mouth and anus. In contrast, vertebrates, together with echinoderms, protochordates and hemichordates, are deuterostomes (Fig. 1), undergoing indeterminate cleavage with the mouth forming some distance from the blastopore which gives rise to the anus (Barnes 1980). The similarities noted in the blood cells of these two groups may represent an example of convergent evolution in which cells from different evolutionary stocks evolve to resemble each other in response to similar evolutionary pressures.

Another example, which clearly illustrates the problem in identifying homologous structures, concerns the lymphocyte- and erythrocyte-like cells of echinoderms. Lymphocyte-like cells of echinoderms not only closely resemble vertebrate lymphocytes ultrastructurally, but they also respond to mitogens, produce antibody-like molecules, interact during immunity and may be produced in lymphoid-like organs (see summary in Ratcliffe *et al*. 1985 and original refs cited below). Certain blood cells of echinoderms also closely resemble the nucleated erythrocytes of lower vertebrates and the early erythroblast stages of mammals (Fontaine & Lambert 1973). In this case, since both the echinoderms and vertebrates are deuterostomes, it is much more difficult to decide whether echinoderm blood cells and associated lymphoid-like organs (Smith 1978, 1980) are truly homologous or analogous with those of lower vertebrates. Not surprisingly, there is disagreement on this point in the literature. Some authors advocate homology (Smith 1978, 1980), others supporting analogy and convergent evolution (Fontaine and Lambert 1973), while others are more cautious and just state that the echinoderm immune system has characteristics resembling those of vertebrate immunity (Leclerc *et al*. 1986). An identical situation is found with the tunicate blood cells, presumably also as a result of their phylogenetic position, with the lymphocyte-like cells regarded as homologous by some (e.g. Wright 1976)

while others believe that such a conclusion is highly questionable (e.g. Warr & Marchalonis 1978). No doubt additional biochemical and molecular analyses will help to clarify matters.

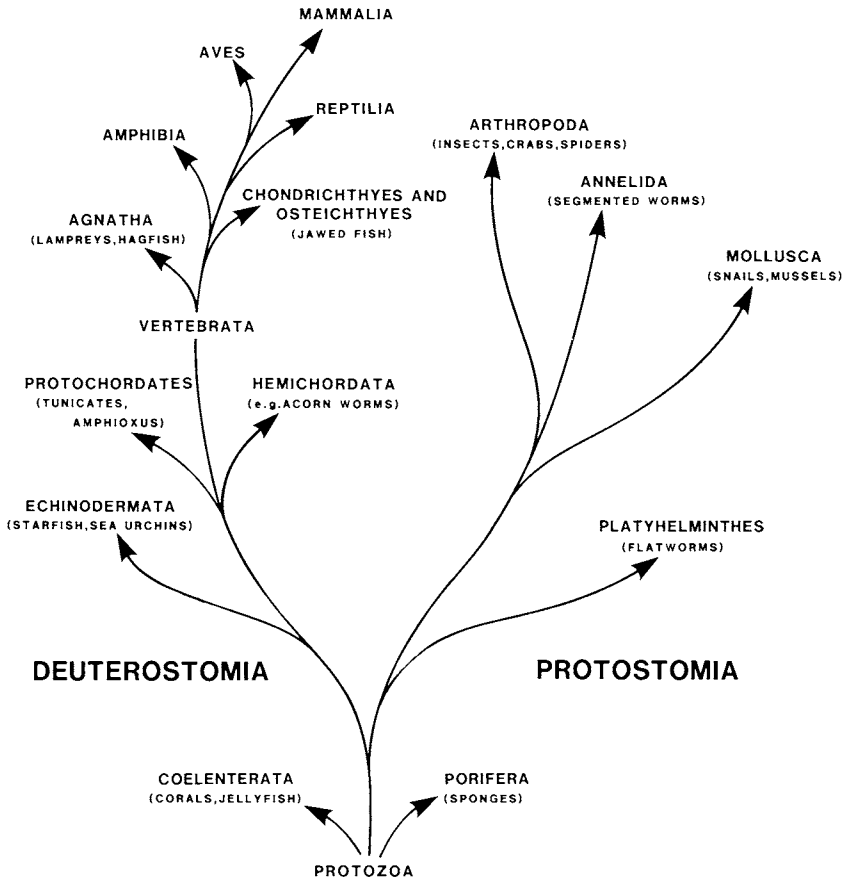

Fig. 1 Phylogenetic scheme for the main groups in the animal kingdom.

A final problem which, as pointed out by Cooper (1976a), continues to frustrate attempts to trace the origins of blood cells concerns the confusing terminology still adopted by many invertebrate haematologists. There is the continuing insistence of using vertebrate terms such as lymphocyte, granulocyte and macrophage to describe invertebrate leucocytes when in actual fact there is little evidence for true homology. Therefore, any vertebrate immunologist hoping to work on the origin and evolution of blood cells will undoubtedly be totally confused by the use of familiar names, such as lymphocyte, to describe cells that are little more than progenitor or stem cells and which occur throughout many of the invertebrate phyla. The situation is further aggravated by the sheer diversity of the invertebrates, together comprising over 95% of all known species in the animal kingdom, and which has resulted in a multitude of names for their blood cells. For example, the invertebrate phagocyte has been variously called an amoebocyte, granulocyte, macrophage, monocyte, plasmatocyte and granular cell (Ratcliffe & Rowley 1979). More recent publications have hopefully clarified invertebrate haematology (e.g. Ratcliffe & Rowley 1981) while, until such time as true homology can be proven, a functional rather than a morphological classification scheme might prove to be most useful (Ratcliffe et al. 1985).

POSSIBLE STAGES AND FACTORS INVOLVED IN THE EVOLUTION OF BLOOD CELLS AND THE IMMUNE SYSTEM

Since the majority of blood cell types are involved in the immune system then consideration of the main steps in the evolution of this system may provide clues as to the origin of the various cell types (Table I).

The first blood cells

All invertebrates are capable of recognising foreignness and effecting phagocytosis. Even the protozoans possess cell surface determinants, exhibit transplantation incompatibilities (so that they can distinguish self from non-self)(Hildemann et al. 1981), and contain lysosomes within their cytoplasm. They thus appear to be independent, wandering, phagocytes, well-suited as potential immunocytes. With the evolution of the Metazoa, possibly from some form of colonial protozoan (Haeckel 1874), organised cell layers first appeared incorporating wandering phagocytic cells, termed archeocytes in sponges. Most, if not all, subsequent animal phyla contain wandering phagocytes which may have arisen from those of the lowly invertebrates by adaptive radiation (Cooper 1976a). Once the third body layer or mesoderm evolved, a cavity (the coelom) developed between the body wall and the gut so that a circulatory system was required to transport trophic and waste substances around the body. At this point in evolution, the blood cells probably migrated from the surrounding connective tissues into the haemolymph so that phagocytes were freed from their food-scavenging role and evolved into an array of leucocyte types with more complex immune reactivity (Cooper 1976b). This stage in evolution can be seen in certain Platyhelminthes where, in some advanced forms, the phagocytes move out of the parenchymatous connective tissue into

TABLE 1 Evolutionary Steps of Possible Significance in the Phylogeny of Blood Cells and the Immune System [a]

	EVOLUTIONARY STEP/PRESSURE	IMMUNOLOGICAL IMPLICATIONS
1.	Protozoa	Recognition and Discrimination
2.	Metazoa Including Colonial Forms	Histocompatibility System, Allogeneic Recognition and Short Term Memory
3.	Mesoderm and Circulatory System. Nutrition and Defence as Separate Functions	Freely Circulating and More Diverse Blood Cell Types. Cellular Immunity and Erythrocytes
4.	Cancer Associated with Increasing Complexity and Longevity	Immunosurveillance
5.	Ancestral Protovertebrate	Increased Recognition and Discriminatory Powers?
6.	Lower Vertebrates with Increased Body Size, Longer Life Span and Reduced Reproductive Potential	True Lymphocytes, Lymphoid Tissue and Antibody Production (IgM), Longer Term Memory
7.	Emergence on to Land, Exposure to Irradiation and Development of High Pressure Blood Vascular Systems	Bone Marrow, Additional Antibody Classes, T- and B- Lymphocytes, Lymphoid Organs with Increased Complexity, GALT
8.	Amniotes (reptiles, birds, mammals) with Loss of Free-living Larval Form	Advanced Differentiation of Immuno-competent Cells Allowing Increased Diversity and Efficiency of Immune System
9.	Homoiothermy with More Favorable Environment for Pathogens	Increased Efficiency of Immune System, Integrated Cellular and Humoral Responses, Germinal Centres in Secondary Lymphoid Organs, Lymph Nodes
10.	Viviparity with Maternal-Foetal Interactions	Additional Fine Tuning of Immune System

[a] Information from Burnet (1968), Marchalonis and Cone (1973), Marchalonis (1977) Manning (1975, 1979, 1980), Manning and Turner (1976), Cooper (1976a,1982) Cooper et al. (1980), Hildemann et al. (1981), Warr (1981).

the haemolymph (Stang-Voss 1974).

The MHC system

Another significant development in the evolution of the immune system also appears in primitive and other invertebrates. Thus transplantation experiments with sponges, coelenterates, annelids, echinoderms and tunicates have shown the presence of allogeneic recognition with a short-term memory component in many of these animals (reviewed in Ratcliffe et al. 1985). It is surprising that even the lowly sponges exhibit alloimmune rejection with specific short-term memory (Hildemann et al. 1979) and that the actual cytotoxic reactivity during allograft rejection involves cytotoxic or "killer" cells crossing over via tissue bridges to destroy the allogeneic cells (Bigger et al. 1981) These results have been interpreted to indicate the presence, in these invertebrates, of an ancestral MHC system with considerable polymorphism at the locus or loci controlling graft acceptance and rejection. Hildemann (1977) therefore proposed that the histocompatibility (H) system underlying cell-mediated immunity is ancestral to and also separate from the immunoglobulin (Ig) system found in vertebrates. At the level of the vertebrates, the Ig system was possibly added to provide a more finely tuned recognition ability in the physiologically complex vertebrates. Such additional regulation would be furnished by the Ig system of circulating antibodies and cell surface receptors (Hildemann et al. 1981). The acquisition of both Ig and H systems has important implications as far as the evolution of blood cells is concerned. In the 'higher' vertebrates, as these two systems become more closely integrated then immune reactivity was more exquisitely controlled and the interactive T- and B-lymphocytes evolved. Indeed, the evolution of the MHC in the vertebrates was an essential pre-requisite to the development of the various T-cell subpopulations with surface receptors for class I (cytotoxic T-cells) or class II (helper T-cells) MHC-encoded proteins.

Further evidence for the emergence of lymphocytes with T-cell properties before those with B-cell properties has been provided by studies on naturally-occurring, dinitrophenyl hapten-binding proteins in the tunicate, Pyura stolonifera. Marchalonis & Warr (1978) showed that these molecules consist of a single subunit with a molecular weight of 65,000- 70,000 daltons and upon electrophoresis resemble the heavy (μ) chains of IgM which is the most primitive immunoglobulin. Further analysis showed that these subunits are of similar mass to the T-cell receptor so that specific cellular immunity may well have evolved before the humoral system.

Various reasons have been postulated for the presence of alloreactivity at such an early stage during evolution in animals as primitive as sponges and corals. Since allogeneic recognition is apparently absent in both molluscs and insects, alloreactivity seems to have been selected for in colonial invertebrates in which transplant rejections may be essential for survival (Lackie 1980; Scofield et al, 1982). The lack of allogeneic and xenogeneic incompatibility in such colonial

forms would soon result in colony-colony overgrowth and fusion with resultant loss of individual integrity. In keeping with this hypothesis, Buss and Green (1985) have suggested that any fusion of allogeneic colonies would cause loss of fitness of one of the partners in the chimaera unless each partner has precisely the same commitment in gamete production. In other words, one partner would be effectively parasitized by the other component of the chimaera.

Cancer

Another force favouring development of the cellular elements of immunity may have been somatic mutation. There is some dispute as to whether neoplasms identical to those of mammals occur in invertebrates although uncontrolled cancer-like growths have been reported in insects and molluscs (Farley 1969; Gateff 1978). It may be significant that of all the invertebrates, these two groups apparently lack allogeneic recognition and therefore the ancestral H system may be poorly developed. With the increase in complexity and longevity of animals during evolution, somatic mutations would constantly occur and, unless a competent immunosurveillance system is present, cancer and premature death will result. As pointed out by Burnet (1968), this would be disastrous if it occurred in the prereproductive period which would be possible if cancer was contagious and could be passed on from old to young individuals. There must, therefore, have been great pressure to develop a surveillance system based upon the recognition of foreign histocompatibility antigens on the surface of a mutated cell by circulating effector cells. The implications of this are obvious as the recognition system involved would have to be extremely finely tuned, to distinguish abnormal from normal body cells, and mediated by specific cell surface receptors. There would thus be considerable evolutionary forces favouring retention and development of the MHC system and of the cellular rather than the humoral components of immunity. This again would favour the diversification of blood cell types, for example, the cytotoxic T-cells involved in immunosurveillance.

Evolution of Vertebrates

Two facts are immediately evident from a comparison of blood cells and immune systems from different vertebrate groups. First, since cyclostomes (hagfish and lampreys) exhibit lymphocyte heterogeneity, possess immunoglobulin-bearing lymphocytes and plasma cells, and produce specific antibodies (Hildemann & Thoenes 1969; Kilarski & Plytycz 1981; Raison & Hildemann 1984; Zapata *et al*. 1984), then the blood cells and immune system of the most primitive vertebrates are considerably more advanced than those of the most highly evolved invertebrates (see below, however, 'lymphocyte-like cells', for the more recent work on echinoderms). Second, comparison between the cyclostomes and mammals shows just how much the blood cells and associated immune processes have evolved within the vertebrates. Thus, mammals exhibit accelerated allograft rejection and specific memory, interactive T- and B-lymphocytes including T-helpers, T-suppressors and cytotoxic T-cells, as well as killer and natural

killer cells, diversity of antibody classes and well defined primary and secondary lymphoid organs.

The main events and forces probably responsible for evolutionary changes in the blood cells and immune potential of vertebrates have been discussed previously (e.g. Manning & Turner 1976; Cooper 1976a; Cohen 1977; Marchalonis 1977). The spleen and thymus first emerge as discrete organs during evolution from cyclostomes to primitive fish, while accelerated allograft rejection first appears in the teleosts, indicating the presence of a modern-type MHC system. Despite these advances, there is still doubt about the presence in agnathous fish of typical mammalian type B- and T-cells although lymphocyte heterogeneity seems to occur in gnathostomate fish (e.g. Miller et al. 1985; see also Chapter 2).

Evolution of the lymphocyte into a number of well-defined cell types seemed to await the emergence of vertebrates from water to land. This single evolutionary step heralded major changes in the blood cells and immune systems of vertebrates. Among the anurans (frogs and toads) we find, mainly for the first time, bone marrow, lymph nodes, gut-associated lymphoid tissues, antibody diversity with both IgM and IgG, and, although debatable, true B- and T-cell dichotomy (eg Borysenko 1976; Du Pasquier 1976; Cooper et al. 1980; Manning & Horton 1982). The appearance of bone marrow is presumably related to the hollow bone structure for locomotion on land and is an ideal site for blood cells which would be protected from harmful ionizing radiation by the hydroxyapatite of bone (Cooper et al. 1980). The reason for the diversity in antibody classes at the amphibian level may be related to the progressive evolution of high-pressure blood vascular systems in terrestrial animals. Changes in the rate of circulation and hydrostatic and osmotic pressures of the blood may make it difficult for the movement of high molecular weight antibody (IgM) into extravascular compartments to reach infectious agents, so that a lower molecular weight antibody (IgG) evolved (Manning & Turner 1976). In 'higher' vertebrates, such as mammals, although as in most vertebrates, IgM acts as the antigen receptor on B-lymphocytes, it is IgG which is capable of binding to monocytes and neutrophils via specific Fc receptors to enhance both the specificity and activities of these cells during phagocytosis and cytotoxicity (Atassi et al. 1984). Thus, many of the activities of the blood cells and of the immune systems of higher vertebrates are controlled at least partially by the phylogenetically recently acquired IgG and IgE antibody classes. The acquisition of a variety of new antibody classes may well have encouraged the diversification of the lymphoid cells of higher vertebrates.

Other important evolutionary steps influencing the increase in efficiency and sophistication of vertebrate immunity and blood cells, include the loss of the free-living larva with protection of the young, and development of homiothermy and viviparity. In amniotes, with the loss of free-living larvae, there is no longer a pressing need to

provide an effective immune system during early stages of development and this may allow a build-up of immunocompetent cell populations before functional commitments are necessary (Manning 1975). The development of homiothermy in birds may also have provided an evolutionary incentive for promptness and diversification in the immune system in order to protect the young from invading microorganisms which would multiply rapidly in the warm and nutritionally rich environment (Manning & Turner 1976). Finally, the development of viviparity in most mammals, during which maternal/foetal interactions occur over long periods, would also have necessitated refinements in the control and feedback mechanisms of the immune system in order to avoid potential rejection processes. The protected environment would also have allowed long periods for the production of immunocyte populations and may have aided in the acquisition of functional heterogeneity of the cells (Manning & Turner 1976).

A COMPARISON OF INVERTEBRATE WITH VERTEBRATE BLOOD CELLS

In this final section, a basic outline of invertebrate blood cell types is given and a brief comparison with vertebrate blood cells is made. The latter are described in detail in the remaining chapters of this book.

Macrophage- and granulocyte-like cells

Since all invertebrates have macrophage- and granulocyte-like cells then phagocytic cells have been conserved throughout phylogeny (Ratcliffe & Rowley 1981; Cooper 1982). In invertebrates, these cells are responsible for phagocytosis and entrap invading metazoan parasites within multicellular capsules too (Ratcliffe *et al.* 1985). Descriptions of encapsulation by granulocytes of metazoan parasites injected into the peritoneal cavity of mammalian species are almost identical to those reported for invertebrate species. For example, when newly encysted juvenile *Fasciola hepatica* are injected intraperitoneally into sensitized rats they rapidly become encapsulated by interactive populations of mast cells, eosinophils and neutrophils (Davies & Goose 1981). This process appears, superficially, almost identical to the granular cell/plasmatocyte co-operation occurring during the encapsulation process in insects (Schmit & Ratcliffe 1977).

Anderson (1981) undertook an interesting comparison of invertebrate and vertebrate leucocytes in which he pointed out that invertebrate leucocytes contain many different enzymes, including acid and alkaline phosphatase, β-glucosaminidase, indoxyl esterase and non-specific esterase. Some of these occur in discrete granules which must therefore be lysosomal and capable of killing and degrading ingested materials such as bacteria. In addition, enzymes are released from these leucocytes and must serve as mediators of protective processes. He concluded, however, that based on lysosomal hydrolases it would be difficult to decide whether invertebrate phagocytes were more like mammalian granulocytes or macrophages although the absence of myeloperoxidase in the invertebrate cells did suggest a closer affinity

to the macrophage cell types. Subsequent work by Sminia et al. (1982), Nakamura et al (1985) and others does indicate, however, that some invertebrate phagocytes do contain the myeloperoxidase system. Anderson (1981) has shown too that, in contrast to vertebrate phagocytes, no Fc or C3 receptors can be detected on invertebrate blood cells, and the metabolic events accompanying phagocytosis by invertebrate leucocytes are fundamentally different from those characteristic of granulocytes and macrophages. Thus, unlike vertebrate phagocytes, the direct oxidation of glucose is not stimulated by phagocytosing invertebrate blood cells. As far as we are aware, this latter conclusion has not been subsequently challenged while more recently Bertheussen & Seljelid (1982) have detected C3 receptors on coelomocyte surfaces in an echinoderm.

Cells resembling mast cells

Cells resembling vertebrate mast cells have been reported in annelids, insects, echinoderms and tunicates in which they are mainly of unknown function (Ratcliffe & Rowley 1979). These leucocytes are termed spherule cells, eleocytes, chloragogen cells, mucocytes, trephocytes, morula cells and vibratile cells, depending upon the animal group in which they occur. Typically, like vertebrate mast cells, the cytoplasm is filled with large inclusions often with a crystalline or microtubular substructure, which appears to contain various mucopolysaccharides. A recent paper by Smith & Smith (1985) emphasises just how similar invertebrate spherule cells are to vertebrate mast cells. Not only do the red spherule cells of the echinoderm, Mellita quinquiesperforata, closely resemble vertebrate mast cells morphologically, but it is significant that spherule cells sensitized with human serum discharge their granules. Furthermore, this degranulation occurred when animals were stressed and, even more compelling, the granules contain significant levels of histamine. The entire spherule cell response resembles that of vertebrate mast cells, which following IgE binding and subsequent degranulation develop a Type I hypersensitivity reaction.

Lymphocyte-like cells

Cells closely resembling vertebrate lymphocytes in morphology have been observed in many invertebrate groups (Ratcliffe et al. 1985). Most attention has been focussed on annelids, echinoderms and tunicates. Research using annelids was stimulated by the discovery of allograft and xenograft rejection in earthworms which exhibited both specificity and short-term memory (Valembois 1963; Duprat 1964; Cooper 1969). These processes together with adoptive transfer, graft infiltration by lymphocyte-like cells, blastogenic response towards transplantation antigens and T-cell mitogens such as PHA and Con A, and the presence of Con A membrane receptors (see refs in Cooper 1981, 1982), are similar to reactions in vertebrates. Blood cells involved in the two groups are, as mentioned previously, probably analogous due to the relative phylogenetic positions of annelids and vertebrates: any similarities may represent an example of convergent evolution. There has also been much interest in the lymphocyte-like cells of