Comparative stem cell biology

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ABSTRACT This review collates data from a range of stem cells studies in an attempt to bring together an overall view of stem cell biology. Data from hemopoietic, keratopoietic, hepatopoietic and neuropoietic stem cells are presented. The developmental and cell biology of each system is discussed in an attempt to develop a comparative view of stem cell biology. Comparisons are drawn in the areas of clonal analysis, surface antigen expression, adhesion molecules and cytokine interactions. Where appropriate the role of embryonic stem (ES) cells is also considered in the developmental biology of the cells in question.

KEY WORDS: *stem cells, hemopoietic, keratopoietic, hepatopoietic, neuropoietic*

Introduction

'Now angrier than before, Zeus had Prometheus chained naked to a pillar in the Caucasian mountains, where a greedy vulture tore at his liver all day, and there was no end to the pain, because every night his liver grew whole again' (Atlas and Prometheus).

The quotation above is perhaps the first ever reference to the activity of stem cells. Modern developmental biologists study stem cells in the ontogeny of tissues and organs such as bone marrow, skin and liver and in self-renewing tissues stem cells can also be studied in the adult organism (Zon, 1995).

Stem cells are defined as cells with extensive self-renewal properties extending throughout the life of an organism and stem cells are therefore present in all renewing tissues (Leblond, 1981). It is this fact that raises the question of common origins and common properties of stem cells in various tissues which will in turn lead to an understanding of what determines the self-renewal or differentiation pathway of stem cells.

This review will assess hemopoietic, epidermal, hepatic and neuronal stem cells in an attempt to bring together the complex data in each area. A consideration of the developmental biology, physiology and cell biology of each type of stem cell may allow a better understanding of the mysterious and fascinating world of stem cells.

Hemopoietic stem cells

Developmental biology

The developmental biology of the hemopoietic stem cell has been studied more than any other stem cell. Early studies showed that the hemopoietic stem cell is first detectable in the mouse yolk sac at day 7 of gestation (Moore and Metcalf, 1970) and that the stem cells then migrate to the fetal liver and differentiate further in that new micro-environment (Hollands, 1987, 1991). Nevertheless, the yolk sac and fetal liver hemopoietic stem cells may be distinctly derived cell populations (Turpen *et al.*, 1981; Wong *et al.*, 1986a,b).

The development of embryonic stem cell (ES) techniques have also helped in the understanding of hemopoietic stem cell biology. Embryonic stem cells (ES) are obtained by culturing cells from mouse blastocysts on STO fibroblast feeder layers (Evans and Kaufman, 1981; Martin, 1981). The resultant cells grow as compact colonies of small cells carrying the stage specific embryonic antigen (SSEA-1) (Solter and Knowles, 1978), and ES cells can differentiate into multiple tissue types *in vitro* in embryoid bodies (Doetschman *et al.*, 1985). The fibroblast feeder layer was found to be producing a substance which allows ES cells to self-renew without differentiation. This molecule has been identified as differentiation inhibitory activity, DIA (Smith *et al.*, 1988) which has subsequently found to be identical to leukemia inhibitory factor, LIF (Gearing *et al.*, 1987). The development of recombinant DIA/LIF has enabled the maintenance of undifferentiated ES cells *in vitro* without the use of feeder layers (Williams *et al.*, 1988).

It is known that ES cells (in the absence of DIA/LIF) can differentiate into nucleated erythrocytes similar to those found in the yolk sac (Doetschman *et al.*, 1985). In these conditions the ES cells evidently change from being totipotent stem cells to committed hemopoietic progenitors. These progenitors can also be directed to produce specific cell lineages *in vitro* by culture with appropriate cytokines (Burkert *et al.*, 1991; Wiles and Keller, 1991). It has also been shown that ES cells can carry out globin gene switching *in vitro* (Lindenbaum and Grosveld, 1990) and that embryoid bodies derived from ES cells contain hemopoietic cells which follow the same developmental sequence of events as in the embryo (Keller *et al.*, 1993). It is therefore possible that ES cells represent true totipotent stem cells from which hemopoietic stem

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cells can be derived and that each lineage sequentially differentiates from this stem cell starting with the erythroid line. Other workers have proposed differentiation models for hemopoietic stem cells (Brown *et al.*, 1988; Nicola and Johnson, 1988) but ES cells may provide a model for the ultimate understanding of the developmental biology of hemopoietic stem cells.

Cell biology

An examination of the current knowledge on the cell biology of the hemopoietic stem cell will enable a better understanding of comparative developmental stem cell biology. The development of *in vivo* and *in vitro* assays for hemopoietic stem cells have allowed the study of hemopoiesis to progress relatively rapidly. The murine *in vivo* colony forming unit-spleen (CFU-S) assay (Till and McCulloch, 1961) and the *in vitro* colony forming cell (CFC) assay (Dexter *et al.*, 1976) enable quantitative and qualitative analysis of hemopoietic stem cell populations. It has subsequently been found that the repopulating ability of a sample of bone marrow is not proportional to the number of CFU-S present. The repopulation of irradiated mice (Chertkov *et al.*, 1985) and the establishment of long term hemopoiesis *in vitro* (Ploemacher and Brons, 1988; van der Sluijs *et al.*, 1990) is not dependent on CFU-S numbers indicating that the CFU-S does not represent the true stem cell.

The discovery of the membrane associated glycoprotein CD34 (Civin *et al.*, 1984, 1989) which is expressed on myeloid progenitor cells (Beschorner *et al.*, 1985) and some endothelial cells (Watt *et al.*, 1987) has enabled the cell biology of hemopoietic progenitor cells to progress rapidly. The CD34 expression in normal bone marrow has been shown to be in the range of 1-3% and this CD34+ cell population contains virtually all of the unipotent progenitor cells (Civin *et al.*, 1984; Katz *et al.*, 1985; Andrews *et al.*, 1986). The CD34+ cell population has been used to repopulate the hemopoietic system of irradiated baboons (Berenson *et al.*, 1988) and rhesus monkeys (Wagermaker *et al.*, 1990) indicating that the pluripotent stem cell must be present in the CD34+ cell population. It has also been shown that clinical autotransplantation of CD34+ progenitor cells is a useful and valuable technique (Berenson *et al.*, 1991) and that in general the number of CD34+ cells transplanted correlates with the hemopoietic recovery of patients following myeloablative therapy (Siena *et al.*, 1991). Successful transplantation of mobilized peripheral blood stem cells (PBSC) (Kessinger and Armitage, 1991) and umbilical cord blood (Wagner *et al.*, 1992) has demonstrated the presence of CD34+ cells in these cell populations. The presence of CD34+ cells in umbilical cord blood is of particular interest to developmental biologists since these cells may represent primitive hemopoietic cells released into the fetal circulation at parturition. Our own studies have shown that umbilical cord blood contains up to 10% CD34+ cells (Hollands and Koncewicz, unpublished data) and studies are underway to assess the numbers of primitive hemopoietic progenitor cells in umbilical cord blood.

A further development in the understanding of hemopoietic progenitor cell biology has been the development of the plastic adherent delta assay (Gordon, 1994). This assay utiliZes the adherent properties of hemopoietic progenitor cells and subsequently assesses the progeny of these plastic adherent cells. It has been shown that human plastic adherent bone marrow cells can generate large numbers of granulocyte and monocyte/macrophage colony forming cells and that this population must therefore contain true myeloid progenitor cells (Gordon *et al.*, 1987, 1989). Other workers have shown that plastic adherent cells can initiate long-term bone marrow cultures indicating the presence of primitive progenitor cells in the plastic adherent population (Kerk *et al.*, 1985). These observations will be important when the common properties of stem cells are considered later in this review.

An important area of study in the comparison of stem cells is the response of stem cells to cytokines. These data may provide information on the common developmental pathways which all stem cells may follow. The hemopoietic stem cell has once more been studied extensively in this context (Moore, 1995). In order to understand the complex subject of cytokine stimulation of hemopoietic stem cells it is necessary to first consider each major cytokine involved and to then develop an overview of the subject.

The interleukin family has a major role to play in the stimulation of hemopoietic stem cells and often have the most notable affects when applied synergistically, especially the combination of IL-1, IL-3 and IL-6 (Ogawa, 1993). Purified CD34+ hemopoietic progenitor cells produce IL-1 mRNA when stimulated with IL-3, IL-6 and Stem Cell Factor (SCF). This suggests that CD34+ cells can produce factors which in turn synergise with other cytokines (Watari *et al.*, 1994). Interleukin 3 and Interleukin 6 appear to have an important synergism when acting on CD34+ cells since IL-3 alone is unable to promote *in vitro* colony formation whereas the two cytokines combined can produce large numbers of colonies of many lineages (Leary *et al.*, 1992). Interleukin 11 has been shown to synergise with IL-3, IL-4 and SCF to produce multilineage *in vitro* colonies (Musachi *et al.*, 1991; Ogawa, 1993) and IL-11 can also synergise with IL-3 or SCF to produce megakaryopoiesis *in vitro* (Yonemura *et al.*, 1992; Du and Williams, 1994).

Leukemia Inhibitory Factor (LIF) has been discussed earlier in this review in relation to the inhibition of ES cell differentiation. LIF has also been shown to decrease the *in vitro* colony formation by cytokine stimulated CD34+ cells and to increase the number of cells with a primitive phenotype. This indicates that LIF inhibits the differentiation of adult, as well as embryonic, stem cells (Brandt *et al.*, 1994).

Stem Cell Factor (SCF) has been referred to earlier and is an important cytokine in the development and regulation of hemopoietic stem cells (Anderson *et al.*, 1990; Huang *et al.*, 1990; Zsebo *et al.*, 1990). SCF alone blocks apoptosis in CD34+ cells but cannot cause non-cycling cells to come into cell cycle (Moore and Hoskins, 1994).

The response of adult or embryonic hemopoietic progenitor cells to cytokines is evidently complex and multifactorial. Current data suggest that the combination of IL-1ß, IL-3, IL-6, SCF, G-CSF and GM-CSF will result in the optimum amplification of peripheral blood CD34+ progenitor cells (Haylock *et al.*, 1992). Nevertheless, other workers claim optimum amplification by omitting G-CSF and GM-CSF from the recipe above and adding erythropoietin (Epo) (Brugger *et al.*, 1993). Human umbilical cord blood CD34+ progenitor cells appear to respond best to the combination of IL-1, IL-3, SCF and Epo (Moore and Hoskins, 1994). The true interactions of the cytokines mentioned, and those still to be discovered, are unclear. However, detailed examination of cytokine specificity and action may assist in the identification of the totipotent stem cell.

Keratopoietic stem cells

Developmental biology

The developmental biology of the true keratopoietic stem cell is poorly understood. The epidermis begins as a single layer of

ectodermal cells which by 4 weeks gestation forms a thin layer of flattened cells known as the periderm (Sengel, 1976). At the end of the first trimester the epidermis has developed into three layers consisting of a mitotically active basal layer, an intermediate layer and a surface periderm. The final structural change occurs at the end of the second trimester when the postnatal epidermis is formed beneath the periderm and the periderm cells are sloughed into the amniotic fluid (Carlson, 1988).

At earlier stages in development, specifically the neural plate at the very early gastrula stage, it has been shown that cells that would normally form the neural plate can be transplanted to the ventral side of the gastrula and in this site will develop into epidermal tissue. If transplanted at a later stage of gastrulation these cells develop into neuronal cells (Spemann, 1918). Evidently the micro-environment of these very early cells directs the differentiation pathway. Other workers have shown that there are possibly growth factors in action during the fetal development of epithelial tissue (Ebba *et al.*, 1980) and that cell surface receptors may be involved in directing cell movement resulting in the formation of define organ boundaries (Hood *et al.*, 1977).

Cell biology

The epidermis of all mammals is maintained at the appropriate thickness by a balance between cell loss at the surface and renewal by proliferation and differentiation of stem cells in the basal layer (Lavker and Sun, 1983). Most of the cells in the basal layer are involved in keratin production (Allen and Potten, 1974) and approximately 85% are in the keratinocyte lineage and are either actively dividing or differentiating to produce keratin (Potten *et al.*, 1978). The balance between proliferative and regenerative cells is not known but it is assumed that the keratopoietic stem cell exists in the minority (Potten, 1976). Radiobiological studies show that 5-10% of the basal cells have regenerative properties following radiation damage (Potten, 1975) and between 2-7% of the basal cells show clonogenic proliferation following radiation damage (Potten and Hendry, 1973; Potten, 1975).

The *in vitro* growth of human keratinocytes has enabled the study of potential keratopoietic stem cells. These systems fall into the following categories: growth on mouse 3T3 fibroblast feeder layers (Rheinwald and Green, 1975) and growth on collagen gels (Karasek and Charlton, 1971), collagen containing fibroblasts (Bell *et al.*, 1981) and growth in the presence of viable or dead dermis (Fusenig *et al.*, 1983; Prunieras *et al.*, 1983). The growth of single keratinocytes on a mouse 3T3 feeder layer has resulted in the clonal analysis of keratinocytes in a comparable system to the colony forming cell of the hemopoietic stem cell (Barrandon and Green, 1987). These experiments have shown that there are three clonal types of keratinocyte which have been termed holoclones, meroclones and paraclones. Holoclones have the greatest growth potential and are likely to contain keratopoietic stem cells, meroclones are a mixed composition of cells, some with unidirectional and some with terminal growth, and paraclones contain short life-span cells (maximum of 15 generations) which often carry the marker involucrin which is a marker of terminal differentiation. Holoclones may convert into meroclones which can in turn convert into paraclones indicating a sequential differentiation pattern for the keratopoietic stem cell.

The role of cytokines in the differentiation of keratopoietic progenitor cells is an important area of study and was concentrated initially on the role of epidermal growth factor (EGF) in the stimulation of epithelial and fibroblast cell lines (Fox *et al.*, 1979; Takenaga *et al.*, 1980). It has since been shown that EGF increases the life-span of keratinocytes *in vitro* without increasing the growth rate (Rheinwald and Green, 1977) and that EGF and transforming growth factor (TGF ß) can stimulate lateral migration of peripheral zone cells in expanding keratinocyte colonies (Barrandon and Green, 1987).

The interleukin family has an important role in the maintenance of keratinocytes and also in the pathogenesis of some skin diseases. Interleukin 1 (IL-1) is produced in large amounts by keratinocytes (Gahring *et al.*, 1985; Kupper, 1989b) and keratinocytes have large numbers of IL-1 receptors on their surface (Kupper *et al.*, 1988b). In response to stimulation by IL-1 keratinocytes produce GM-CSF, IL8 (Larsen *et al.*, 1989) and IL-6 (Kupper, 1989a). Interleukin 3 (IL-3) has been shown to be produced by murine keratinocytes (Luger *et al.*, 1988) and interleukin 6 (IL-6) has been shown to undergo multiple interactions with other cytokines in the normal physiology and pathology of keratinocytes (Wong and Clark, 1988; Kreuger *et al.*, 1990; Sehgal, 1990). Our own data show that IL-6 is capable of stimulating keratinocyte proliferation *in vitro*, indicating a tentative link between the cytokine response of hemopoietic and keratopoietic progenitor cells (Battersby and Hollands, unpublished).

The production of colony stimulating factors (CSF), normally associated with the regulation of hemopoietic cells, by keratinocytes is an important area of comparative biology. The production of GM-CSF, G-CSF and M-CSF by cytokines is well established (Kupper, 1988a) and subsequent work has concentrated on keratinocyte derived GM-CSF (Clark and Kamen, 1987). GM-CSF has been shown to be important in the proliferation of the macrophage lineage (Chodakewitz *et al.*, 1987) and in the *in vitro* maturation of freshly isolated Langerhans cells by either inducing mature cells to proliferate or inducing maturation of cells *in situ* (Witmer-Pack *et al.*, 1987).

The *in vitro* adhesion and migration of keratinocytes has been studied in some detail, especially the migration of keratinocytes cultured on collagen, thrombospondin and fibronectin (O'Keefe *et al.*, 1985; Nickoloff *et al.*, 1988; Woodley *et al.*, 1988; Guo *et al.*, 1990). It has also been shown that laminin and vitronectin inhibit directional migration of keratinocytes (Nickoloff *et al.*, 1988; Brown *et al.*, 1991). The nature and expression of cell surface adhesive receptors called integrins are evidently important in dictating the adhesive properties of keratinocytes (Hynes, 1992). Beta-1 integrin (CD29) is of particular importance in cell-cell adhesion and has been shown to be concentrated at the cell-cell boundaries of keratinocytes *in vitro* (Carter *et al.*, 1990; DeLuca *et al.*, 1990; Adams and Watt, 1991). Our own studies have shown that purified CD29+ keratopoietic cells preferentially adhere to collagen IV *in vitro* (Hollands *et al.*, unpublished).

It is possible that further study on the role of integrins in cell adhesion will allow a better understanding of the microenvironment of both hemopoietic and keratopoietic stem cells.

Hepatopoietic stem cells

Developmental biology

The developing liver first becomes evident at day 9-10 in the rodent and the 5-somite stage in the human (Croisille and De Louarin, 1965; Clearfield, 1985). The primary hepatic rudiment appears as a thickening in the endoderm of the ventral floor of the

foregut. The hepatic diverticulum and the ventral pancreatic diverticulum arise from the foregut and the hepatic diverticulum moves anteriorly to invaginate into the splanchnic mesoderm of the septum transversum (DuBois, 1963). The hepatic diverticulum then divides into the caudal lobe, which gives rise to the gall bladder, cystic and bile ducts, and the cranial lobe which ultimately will become the liver (Elias and Schrick, 1969). It has recently been shown that hepatoblasts containing α-fetoprotein and albumin can give rise to intra- and extra-hepatic bile ducts (Germain *et al.*, 1988a) and that these cells *in vitro* can differentiate along either hepatocytic or biliary lineages (Germain *et al.*, 1988b). These cells may represent early hepatopoietic stem cells and it has also been suggested that there may be a common stem cell giving rise to liver and pancreas and perhaps a common endodermal stem cell which would also give rise to intestine (Tatematsu *et al.*, 1985; Bisgaard and Thorgeirsson, 1991).

Hepatoblasts may also be assessed by their expression of cytokeratins 8 and 18 and by the presence of cytokeratins 7 and 19 in newly formed ducts (van Eyken *et al.*, 1988; Shiojiri *et al.*, 1991). It is of interest that human hepatoblasts have been shown to express cytokeratin 19 (Gerber and Thung, 1992; van Eyken and Desmet, 1992) which is also expressed on adult keratopoietic cells. Cytokeratin 19 is no longer detectable beyond 14 weeks of gestation and mature adult hepatocytes express cytokeratin 8 and 18, however, bile duct cells continue to express cytokeratins 7, 8, 18 and 19 (van Eyken, 1992). It is also important to note that the oval antigens OC2 and OC3 are expressed on both hepatopoietic and hemopoietic cells indicating a possible common origin for these two cell types (Hixson *et al.*, 1990; Faris *et al.*, 1991).

Cell biology

The human liver has a great capacity for regeneration following physical or chemical damage and this capability evidently indicates the presence of hepatopoietic stem cells (Wilson and Leduc, 1958). Neverthless, there is currently much debate on the nature of hepatopoietic stem cells (Sell, 1990). Studies on hepatocellular carcinoma and cholangiocarcinoma suggest that these diseases arise from pluripotent hepatopoietic stem cells (Sell and Dunsford, 1989). These conditions may provide the model for the study of the hepatopoietic stem cell.

There are currently three main candidates for the putative hepatopoietic stem cell including AE1+ 'biliary' cells (Davies *et al.*, 1990), oval cells (Sigal *et al.*, 1994) and Ito cells (Ito and Menoto, 1952). Extensive studies, mainly in the area of hepatic carcinogenesis, indicate that the oval cells most likely represent the hepatopoietic stem cells (Farber, 1984; Evarts *et al.*, 1987a,b, 1989; Fausto, 1990). These studies show that animals exposed to a wide range of carcinogens develop hepatocellular carcinoma. In these tumors there is an initial proliferation of small, periportal cells with scant cytoplasm and ovoid nuclei, these are oval cells (Germain *et al.*, 1985, 1988; Radaeva-Pronina and Faktor, 1990). It is interesting from a developmental viewpoint to note that oval cells, although they have bile duct cell morphology, have a biochemical profile more closely related to fetal hepatocytes (Hayner *et al.*, 1984; Sirica *et al.*, 1990) and that transitional cells between oval cells and hepatocytes have been identified (Sell, 1980).

The role of cytokines and humoral factors in the regeneration of liver after partial hepatectomy has been described (Higgins and Anderson, 1931; Moolten and Bucher, 1967) in particular hepatocyte growth factor (HGF) (Nakamura *et al.*, 1989; Nakamura, 1991). HGF has been shown to be active in stimulating growth of hepatocytes *in vitro* (Zarnegar and Michalopoulos, 1989) and to be important in liver cell proliferation both *in vitro* and *in vivo* (Ishiki *et al.*, 1992; Shiota *et al.*, 1992). Despite this role in relation to hepatocytes HGF can act on several cell types and indeed the HGF receptor can be detected on a range of cells (DiRenzo *et al.*, 1991; Rubin *et al.*, 1991). HGF has also been reported to act as a 'scatter factor' stimulating cell motility (Furlong *et al.*, 1991; Weidner *et al.*, 1991) and there is evidence that human embryonic lung scatter factor and HGF are the same molecule (Konishi *et al.*, 1991) indicating a developmental importance for HGF. Other workers have shown that HGF can inhibit cell growth, including the growth of hepatocellular carcinoma cell (Higashio *et al.*, 1990; Tajima *et al.*, 1991). HGF is evidently a highly complex molecule which has a variety of physiological and pathological roles, many of which are poorly understood.

Recent attention has focused on the role of insulin-like growth factor-binding proteins (IGFBP) (Lee *et al.*, 1994) and the expression of the IGFBP gene during liver regeneration. It has been shown that the gene encoding for IGFBP is activated within one hour of hepatectomy (Mohn *et al.*, 1991) indicating that such molecules may interact with other cytokines during hepatopoiesis.

A further link between hepatopoietic stem cells and those discussed earlier is an observed preferential adherence of human AE1 cells to tissue culture plastic. We have shown that human AE1+ cells attach to tissue culture plastic with maximum adherence at 40 minutes culture time (Hollands and Hobbins, unpublished data). These cells are candidate hepatic stem cells and may represent the equivalent of the delta cells of the hemopoietic system as discussed earlier.

Neuropoietic stem cells

Developmental biology

The primary formation of the nervous system occurs with the development of the neural plate overlying the notochord and the subsequent folding of the neural plate to form the neural tube (Bergquist, 1952; Alvarez and Schoenwolf, 1992). True multipotential neuropoietic stem cells have been proposed to arise in early neuroepithelium, to undergo mitosis, and to mature into bipotential progenitors which can give rise to either neuronal or glial progenitor cells (O'Rahilly and Gardner, 1974; Cameron and Rakic, 1991). Neuronal progenitor cells give rise to a series of neuroblasts which will ultimately form the mature neuron. Glial progenitor cells produce three major cells lines: 02A progenitor cells which give rise to oligodendrocytes and type-2 astrocytes, type-1 astrocyte progenitors which give rise to type-1 astrocytes and the radial progenitor cell which gives rise to the radial glial cells (Cameron and Rakic, 1991). Radial glial cells are thought of as 'guide wires' in the brain for the migration of young neurons (Purves and Lichtman, 1985; Rakic, 1988).

The developmental biology of the CNS has more recently been studied in relation to embryonic stem (ES) cells (Evans and Kaufman, 1981; Martin, 1981). The culture of ES cells on a suitable substrate in the presence of retinoic acid results in up to 30% of the cells developing neuronal markers and morphology (Bain *et al.*, 1995). Other cells show properties of reactive astrocytes and possibly mesodermal characteristics. Those cells with neuronal

properties have been shown to express neurofilament and microtubule proteins associated with mature neurones and to show similar electrophysiological properties to mature neurones (Bain *et al.*, 1995).

Epidermal growth factor (EGF) has been shown to have proliferative effects on neural cells isolated from embryonic mouse striatum (Reynolds *et al.*, 1992) and from embryonic rat striatum and mesencephalon (Svendsen *et al.*, 1995). EGF responsive cells have also been shown to proliferate in the presence of transforming growth factor (TGF β) and can, on a polyornithine substrate, produce colonies of cells expressing nestin which is a candidate marker for neuropoietic stem cells (Fredricksen and McKay, 1988; Lendahl *et al.*, 1990).

In addition to the role of EGF current work assessing the role of basic fibroblast growth factor (bFGF) has shown it to have a proliferative effect on nestin positive rat striatum cells (Catteneo and McKay, 1990), embryonic cerebral hemispheres (Gensburger *et al.*, 1987), hippocampus (Deloulme *et al.*, 1991; Ray *et al.*, 1993) and spinal cord (Ray and Gage, 1994). The role of growth factors in embryonic neuropoiesis is evidently complex and multifactorial.

Cell biology

The identity and properties of the neuronal stem cell are currently undefined but many studies have been made of candidate neuronal stem cells *in vitro*. Studies on embryonic rat cortex transduced with the β-gal gene have shown the development of colonies of cells expressing the marker gene (Price *et al.*, 1987). These colonies evidently arise from a single β-gal expressing cell within the general cell population. Subsequent studies have shown that approximately 18% of the cells within the rat embryonic cortex can differentiate into neurons and oligodendrocytes *in vitro* (Williams *et al.*, 1991).

Immortalized neuronal cells, transduced with oncogenes, have been used in the study of neuropoietic stem cells (Cepko, 1988, 1989; Lendhal and McKay, 1990). Nevertheless, these cells have been shown to have altered protein expression (Birren and Anderson, 1990; Renfranz *et al.*, 1991; Vandenberg *et al.*, 1991; Whittemore and White, 1993) and a more rapid growth rate than normal primary neuronal cells. These characteristics are an indication that these cells may yield spurious data and indeed have been shown to be indicative of an abnormal karyotype (Bianchi *et al.*, 1993).

Studies on primary precursor cells developing in the neural crest have shown that single neural crest cells are multipotent and these cells may therefore represent stem cells (Stemple and Anderson, 1992). The same study showed that neural crest clones, established on fibronectin and overlaid with poly-D-lysine at various times, produced neurone-only in preference to glia-only clones. The substratum evidently influences the lineage decisions of neural crest stem cells (Stemple and Anderson, 1992). Glial growth factor has been shown to suppress neuronal differentiation, illustrating the additional importance of growth factors in stem cell differentiation (Shah *et al.*, 1994).

There are three types of glial cells found in the optic nerve of the rat, type 1 and type 2 astrocytes and oligodendrocytes. It has been found that type 2 astrocytes and oligodendrocytes arise from a common progenitor in the rat neonatal optic nerve called O-2A (Raff *et al.*, 1983). These bipotential progenitor cells have been demonstrated in adult optic nerve but have different morphological and cell cycle properties to perinatal O-2A cells (Wolswijk and Noble, 1989). It is possible that neonatal O-2A cells are slowly replaced by adult progenitor cells, an idea that is supported by the observation that perinatal O-2A cells when grown long-term *in vitro* express the O4 antigen found on adult progenitor cells (Wren *et al.*, 1992) and that O4 expression *in vivo* increases with age (Wolswijk *et al.*, 1990).

The bipotential O-2A progenitor cell can potentially be used to replace myelin in experimental models (Blakemore and Franklin, 1991). The numbers of O-2A progenitors can be amplified by culture with platelet derived growth factor (PDGF) and basic fibroblast growth factor (bFGF) (Bogler *et al.*, 1990). On transplantation to the spinal cord, O-2A cells differentiate mainly into oligodendrocytes (99%) and the rest differentiate into astrocytes (Crang *et al.*, 1992). These oligodendrocytes are capable of remyelinating up to 90% of axons in a damaged site.

The therapeutic transplantation of neuropoietic stem cells to either remyelinate or reconnect damaged areas of the CNS is an area of intense investigation (Bjorklund, 1994). The possibility of repairing spinal cord damage has been supported by work showing repair of damaged rat spinal cord (Iwashita *et al.*, 1994) thus illustrating the enormous potential in the manipulation of neuropoietic stem cells.

Summary

On first examination the data presented in this review may seem unrelated and of too wide a scope to be generally of interest. Nevertheless, on closer examination it can be seen that the data on the different stem cells do inter-relate and when looked on as a whole can be brought together as a coherent subject. This review attempts to rationalize and standardize current data on stem cells and to bring together experts in different fields. Such collaboration and understanding across disciplines will further the development of stem cell biology. The current "specialization" of stem cell workers inhibits this interchange, whereas a multidisciplinary approach will enable the subject to be viewed as a whole and take insights from what are currently thought of as unrelated areas. The data presented in this review supports the idea that a multidisciplinary approach to stem cell biology can enhance the understanding of interrelationships between stem cells. The traditional clonal assays of the hemopoietic system are reflected in the clonal assays of the keratopoietic system, indeed all stem cells should in theory be capable of producing clonal growth if the growth conditions are correct. The committed progenitor cells of each population carry a wide range of surface antigens and the true stem cell of each population has yet to be firmly identified. The hemopoietic stem cell has perhaps come closest to identification but even in this greatly studied area uncertainties exist. It is possible that each system is maintained by small numbers of true stem cells which divide infrequently and that day to day production of cells in a system is via relatively large numbers of rapidly dividing committed progenitor cells. This idea is supported by the fact that stem cells are difficult , if not impossible, to identify whereas committed progenitor cell populations can be identified relatively easily. Stem cells appear to have a common property of adhesion when cultured *in vitro* either to tissue culture plastic or to coated surfaces. This may reflect the need for stem cells to create a microenvironment to

support normal growth and differentiation. Hemopoietic progenitor cells attach to tissue culture plastic, keratopoietic to collagen IV and hepatopoietic to tissue culture plastic indicating the possibility of similar adhesion molecules being shared between stem cells of different systems. The response of stem cells of different systems to a range of cytokines further illustrates the common properties of these cells. Stem cell factor (SCF) can stimulate hemopoietic and keratopoietic stem cell division in synergism with IL1, 3 and 6 and IL6 respectively. Epidermal growth factor (EGF) can stimulate the differentiation of both keratopoietic and neuropoietic stem cells, indicating similarities in receptors and perhaps ontogeny of these two diverse cell types. The interaction of cytokines with stem cells is a relatively new science but it re-emphasizes the similarities between stem cells. The developmental biology of stem cells has been greatly advanced by studies using embryonic stem (ES) cells. Differentiation of all four tissues cited in this review has been demonstrated from ES cells indicating the common origin of these cells and pointing towards the ultimate stem cell: the fertilized oocyte. Stem cell biology is in its infancy. Ten years ago the number of publications per year in this field was less than one hundred, today this figure is closer to thousands and constantly increasing. Exciting new concepts appear regularly such as the discovery of the mitotic clock or telomeric DNA in stem cells which may lead to an understanding of the ageing process (Vaziri *et al.*, 1994), the observation that CD34+ myeloid progenitor cells can be directed towards lymphoid differentiation *in vitro* (Freedman *et al.*, 1996) and that hemopoietic stem cells have been identified in the adult liver indicating close interactions between these two tissues (Taniguchi *et al.*, 1996). Stem cell biology evidently has a great future in the understanding of development, normal differentiation ageing and ultimately death.

> *"A complete, consistent, unified theory is only the first step: our goal is a complete understanding of the events around us, and of our own existence"* (Hawking, 1988).

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