

REVIEW

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Regenerative biology: the emerging field of tissue repair and restoration

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Abstract Regenerative biology has now been recognized as a new field with certain aims and goals. One direction of this new field is to understand the basic mechanisms by which tissues can be repaired and restored. The other direction examines the possibility of using this basic knowledge to apply it to medicine with the goal to clinically repair damaged tissues. Regeneration of tissues can occur by the differentiation of stem cells (local or non-local) or by the transdifferentiation of local terminally differentiated cells. While the transdifferentiation aspects are old, during the past few years many data have accumulated regarding the existence of stem cells and their participation in tissue renewal. This review will present an overview of the potential of all vertebrate organs to regenerate and of the basic mechanisms involved.

Introduction

Imagine the situation where you can repair any damaged tissue or organ by regenerating a new one from the old or from multipotent reserve cells in your body. That would certainly be the ultimate therapy for many diseases and result in a much better quality of your life. Fiction? Not quite. Regeneration of body parts has fascinated humans (and scientists) since the beginning of civilization. More than two hundred years ago and long before we knew anything about developmental biology, regeneration of body parts in invertebrates and in amphibia was documented. Since then, we have come a long way and now we are facing a new revolution in

medicine: The emergence of Regenerative Biology and Medicine. This is one of the best examples to illustrate how basic biology set the foundations of a new way to attack medical problems and establish new therapies. In this review, I plan to present to the audience a comprehensive synthesis of the ability of the different adult tissues and organs to regenerate or repair when damaged. The basic mechanism for each case will be outlined and the factors involved (when known) will be mentioned. The aim of this review is to leave the reader with an appreciation of the huge potential for repair in vertebrates (including human) and of the issues that are involved. That is why this review will not deal with the impressive regenerative abilities in invertebrates, which can in fact regenerate whole body segments. It is hoped that the reader will appreciate that covering all these issues in details in a review is unrealistic. Therefore, I have concentrated on the basic concepts and main issues only.

The terms tissue repair and regeneration are used sometimes indiscriminately and they basically refer to the same thing. They both can be used to describe how a particular tissue or part of an organ (consisting of two or more tissues) are reconstructed after damage, but these terms should not be confused with wound healing. In wound healing the wound is closed by collagenous scar tissue without restoration of the missing part (there are exceptions, however, see section on skin regeneration). In contrast, during tissue regeneration, the original damaged tissue is restored by a quite faithful copy. More complex regeneration of whole body parts such as seen in invertebrates, in the amphibia (limbs, tail, jaw) and fish (fin), is historically called epimorphic regeneration (Tsonis, 1996).

Regeneration occurs by primarily two strategies. One is by differentiated cells neighboring the damaged site. These cells restore the damaged tissue by proliferation or by transdifferentiation. By transdifferentiation we mean

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that the local cells are able to dedifferentiate (lose the characteristics of their origin) and subsequently redifferentiate. This strategy is used in many cases, such as liver and pancreas, and is characteristic of epimorphic regeneration as well (Tsonis, 2000). The second strategy is by stem cells. Stem cells can be local (tissue-specific; reside in certain adult tissues) and upon damage they differentiate to reconstitute the lost part. Examples of tissue-specific stem cells include brain, skeletal muscle, skin, hematopoietic, and liver cells. Alternatively, there are stem cells that are not tissue-specific. These cells reside in the bone marrow and they can migrate to the damaged site and differentiate to many other cell types. Such stem cells have been found to differentiate to nervous tissue, skeletal muscle, cardiac muscle, liver, and to cells of the mesenchymal lineage. Sometimes the terms stem cells, progenitor cells, reserve cells, or germ cells are used when referring to cells with multipotent differentiation ability (Blau et al., 2001). In Table 1, terminology is provided to clarify the issue. The possibility that stem cells can be used to repair virtually all tissues has recently received enormous attention and is heralded as the one that ultimately would lead to therapies for many diseases. The main challenge here is how to isolate and stimulate stem cells to differentiate as needed. This is very promising and at the present time is the major tenet of the field of Regenerative Biology and Medicine. However, the study of regenerative processes by local differentiated cells should provide us with powerful tools as well and should be an important component in this new field. We should not undermine the fact that the most spectacular cases of regeneration in vertebrates do occur by local non-stem cells. This review will help establish the importance of these two strategies for regeneration in different tissues and organs. In the last section some

Table 1 Terminology

<i>Totipotent Cells:</i>	Cells that are able to give rise to a whole organism. Such cells are the fertilized ovum or cells before the formation of blastocyst.
<i>Embryonal Stem Cells (ES cells):</i>	Pluripotent cells derived from the inner cell mass of the blastocyst, they are able to give rise to any cell type in the body.
<i>Stem Cell:</i>	A cell that is capable of giving rise to different cell types upon selective activation. In this sense a stem cell is pluripotent.
<i>Progenitor Cell:</i>	Arises from a stem cell that has been activated and is committed to a particular lineage only. Such cells are also called <i>reserve</i> cells, especially when they are tissue-specific adult stem cells. Obviously, the degree of differentiation of a progenitor cell is more limited than a stem cell.
<i>Germ Cell:</i>	A differentiated stem cell that is destined to become mature sperm (male) or oocyte (female).
<i>Primordial Germ Cells:</i>	The precursors of germ cells, which migrate from the allantois (in mammals) to the gonads during embryogenesis. When PGCs are isolated while migrating they could be potential totipotent stem cells.
<i>Satellite Cell:</i>	A cell that gives rise to skeletal muscle. It is believed that satellite cells arise by activation of muscle stem cells.

discussion is also attempted to show possible similarities in both strategies.

Nervous system

The nervous system is divided into the central nervous system (CNS) and the peripheral nervous system (PNS). As it is well known, the nervous system has very poor regenerative capabilities after injury. The problem is that axonal and dendritic connections cannot regenerate correctly. In general, however, axons regenerate much better in PNS than in CNS. This might be due to the different affinity of cells, especially the extrinsic supporting cells. Also the composition of myelin seems to be very important (Pestronk et al., 1990). CNS white matter is selectively inhibitory for axonal outgrowth. When CNS axons are grafted in peripheral nerves, they can extend for long distances. One of the paramount molecules that is part of the white matter of CNS is nogo, a reticulon protein that is a potent inhibitor of axonal regeneration. Nogo is expressed in oligodendrocytes, and treatment with antibodies against Nogo results in axonal regeneration and functional recovery after spinal cord injury (Chen et al., 2000; Grandpre et al., 2000).

Some urodele amphibia and fishes are the only vertebrates that can regenerate their brains and spinal cord after injury or transection. Regeneration is accomplished by the differentiation of the ependymal cells. Ependymal cells form a dedifferentiated mass of cells, called blastema, and then these cells differentiate back to ependymal cells (Goss, 1969). As mentioned above, in mammals the ability of CNS for regeneration is very poor. Attempts to induce regeneration after spinal cord injury have mainly focused on the use of neurotrophic or growth factors that are capable of inducing axonal growth. Such molecules include NGF, BDNF, GDGF, CNTF, FGF, IGF, PDGF, and VEGFa (Irving and Mason, 1999; Oudega and Hagg, 1999; Horner and Gage, 2000). Also, TGF-beta, EGF, and inflammatory cytokines, such as LIF or TNF have been shown to have roles in axonal differentiation as well as neurotrophic activity (Horner and Gage, 2000). Strategies for medical applications deal mainly with artificial substrates seeded with growth factors. Some studies have shown successful hindlimb function after complete spinal cord gaps were bridged with grafts and substrates with growth factors (Cheng et al., 1996). Obviously, the identification of factors that do not allow regeneration in the CNS will be beneficial in treating lesions in the spinal cord. Comparative studies with animals that can readily regenerate severed spinal cord or brain should be of value to complement research in mammals.

The other line of hope for CNS regeneration comes from stem cells. Studies several years ago indicated that, against the dogmatically held opinion, new neurons could be formed. In recent years, the presence of neur-

onal progenitor cells in mammals has been confirmed and they can now be routinely isolated and cultured from adult brain (Brustle, 1999; McKay, 1997). Such cells have also been isolated and cultured from human brains even after death (Palmer et al., 2001). Tissue-specific neuronal stem cells can give rise to neurons, astrocytes, and oligodendrocytes, when injected into the brain (Morrison et al., 1999; Gage, 2000; Qian et al., 2000; Van der Kooy and Weiss, 2000). Ependymal cells have been shown to be neural stem cells. They are capable of giving rise to rapidly dividing cells that migrate to the olfactory bulb, a site of extensive regeneration, and also in response to spinal cord injury, ependymal cells proliferate and participate in scar formation (Johansson et al., 1999). Neural stem cells reside also in the subventricular zone. This region generates new neurons destined for the olfactory bulb and is composed of migrating neuroblasts, immature precursors, astrocytes, and ependymal cells (Doetsch et al., 1999). Some of these neural stem cells can also differentiate to muscle when co-cultured with a muscle cell line or into blood, indicating that neural stem cells can also generate non-neural cells (Rietze et al., 2001; Bjornson et al., 1999). In addition, bone marrow-derived cells can give rise to neuronal cells. Such cells have been shown to home in the olfactory bulb and in the hippocampus in the brain (Eglitis and Mesey, 1997; Kopen et al., 1999; Rietze et al., 2001). Replacement therapies with stem cells might be beneficial to diseases such as Alzheimer's, Parkinson's and Huntington's. It is believed that in these neurodegenerative diseases the lost neurons are not replaced, whereas in normal brain endogenous stem cell populations replace cells in lesions (Armstrong and Barker, 2001).

Hair cells

Sensory organs, such as the ones that transmit sound (hair cells) or visual images (lens, retina) have an impressive regenerative ability. Hair cells serve as mechanoreceptors for hearing, balance, and orientation. Hair cells are surrounded by supporting cells and form synapses with the VIIIth cranial nerve, which is responsible for sending the messages to the brain. Hearing loss in humans is manifested by abnormalities and damage in the hair cells and the VIIIth nerve and is irreversible. Some non-mammalian vertebrates, such as amphibia, fishes and birds can, however, produce hair cells throughout their lives. Hair cells are produced mitotically by the vestibular epithelium. Experimentally, regeneration of the hair cells can be achieved by damaging them by ototoxicity or antibiotics. In birds, where much of the studies have concentrated, the hair cells seem to be produced by the supporting cells, which upon damage of the hair cells, reenter the cell cycle and differentiate into new hair cells. In this sense, the supporting

cells are the best candidates for avian hair cell progenitors. The potential mitogens for these progenitor cells are FGF and IGF. These factors and their receptors are expressed in the supporting cells. In frogs and chickens, there is mounting evidence to indicate that hair cells are produced by nonmitotic conversion (transdifferentiation) of the supporting cells (Baird et al., 2000; Stone and Rubel, 2000). The reason why mammals cannot regenerate their hair cells might lie in the fact that in mammals all cells in the auditory organ of Corti become terminally mitotic very early in development. This, however, is not the case in birds despite the anatomical and functional similarities of their auditory epithelia with the mammalian counterpart. This divergence might be due to the nature of the progenitor cells. In this sense, understanding the biology of the avian progenitor cells might provide crucial clues that can be applied mammals and humans. However, a different line of research has shown that mammalian auditory hair cell regeneration is possible by treatment with retinoic acid (Levebvre et al., 1993). Also, disruption of the gene for cyclin-dependent kinase inhibitor p27^{Kip1} allows proliferation in the adult organ of Corti (Lowenheim et al., 1999).

Lens and retina

Some amphibia possess remarkable abilities to regenerate their lens and retina upon removal (Tsonis, 2000). Lens regeneration can be achieved by the pigment epithelium cells (PECs) from the dorsal iris (in some urodeles) and by the inner epithelium of the cornea (in frogs) by the process of transdifferentiation. In urodeles, after lentiectomy, PECs dedifferentiate, lose their tissue characteristics by depigmenting, reenter the cell cycle, and then transdifferentiate to lens epithelial cells. Lens epithelial cells form the lens vesicle and differentiate to lens fibers in a similar manner as during lens development. While only PECs from the dorsal iris can participate in this process *in vivo*, all the cells from the neural pigment epithelium can transdifferentiate to lens cells when cultured. The same is true when these cells are isolated from many other animals even from aged humans. Here we have an interesting phenomenon where the capacity for transdifferentiation exists in all animals, but *in vivo* this capacity is only materialized in some urodeles. Stem cells or progenitor cells do not play roles in this event. The process of lens regeneration is clearly carried out by the transdifferentiation of the PECs from the dorsal iris. Research in this field is concentrated on identifying the factors that make only the dorsal iris capable of transdifferentiation. Identifying these factors and future applications in other animals could lead to therapies for cataracts. Factors that seem to be preferentially expressed in the dorsal iris include the transcriptional factors pax-6 and prox-1 and the receptors for some FGFs, FGFR-1 (Del Rio-Tsonis et al.,

1995; 1997; 1998; 1999). Retinoic acid, which is normally produced in the eye, seems to be important in lens regeneration as well. Blockage of the receptors for retinoic acid has been found to induce ectopic lens regeneration elicited from other sites than the dorsal iris (Tsonis et al., 2000). In mammals, the only successful case of lens regeneration has been reported in rabbits, by leaving the lens capsule after lentectomy (Gwon et al., 1993).

Retina regeneration is also possible by transdifferentiation in urodele amphibia (Mitashov, 1996). Upon retinectomy, the neural retina pigment epithelial cells dedifferentiate, divide, and reconstruct the lost retina. In the newt retina, however, PECs also renew themselves, and because of their ability to transdifferentiate to several cells, they can be regarded as stem cells. Experiments in chickens have shown that retina regeneration is only possible if some portion of the retina is left behind. The retina is regenerated by the PECs and is of reverse polarity. If the whole retina is removed, then the only factor that can restore the retina is FGF (Park and Hollenberg, 1989). In frogs, chicken, and other animals, the retina can be regenerated by the differentiation of multipotent retinal progenitor cells (RPCs) found in the ciliary body (Mitashov, 1996; Fischer and Reh, 2000). The molecular mechanisms mediating the differentiation of RPCs have mainly pinpointed the transcriptional factor *pax-6*. *Pax-6* can control the transcription of other factors, which in turn are responsible for inducing subsets of RPCs to differentiate to ganglion cells, bipolar cells, amacrine cells, horizontal cells, photoreceptor cells, and Muller glia cells. If *pax-6* is inactivated, the potential of RPCs is restricted to one cell fate: that of amacrine cells (Marquardt et al., 2001). Understanding the mechanisms of retina regeneration would be of enormous importance in several degenerating diseases. Activating the PE to transdifferentiate to retina, for example, might lead to experimental or even clinical applications in cases of diseases such as macular degeneration.

Skeletal muscle

Skeletal muscle has a remarkable ability to regenerate after trauma or in response to exercise. However, the response to exercise is rather hypertrophy (enlargement of muscle fibers) rather than hyperplasia (proliferation of muscle fibers). For a long time, muscle satellite cells have been considered as the main reserve cells contributing to muscle regeneration. Satellite cells are mononucleated and are ensheathed under the basal lamina that covers the muscle fibers. Upon injury, satellite cells are activated, initiate cell proliferation, and produce the so-called myogenic precursor cells, which undergo several rounds of proliferation and then fuse to form myofibers. Satellite cells adhere to the surface of the myotubes (Grounds, 1999; Best and Hunter, 2000; Hawke and Garry, 2001). Quiescent satellite cells do not express my-

ogenic regulatory factors, but when they are activated they express either *Myf5* or *MyoD* followed by co-expression of these two factors. Following proliferation, myogenin and *MRF4* are expressed in cells beginning to differentiate (Sabourin and Rudnicki, 2000; Seale and Rudnicki, 2000). Apart from satellite cells, however, tissue-specific stem cells and bone marrow-derived stem cells have also been implicated in skeletal muscle regeneration. The relationship between satellite and muscle stem cells (MuSCs) is not clear. Although it is believed that satellite cells are the MuSCs, MuSCs might be developmentally in a stage before satellite cells. The other possibility is that MuSCs are independent from satellite cells. *Pax 7* is a transcriptional factor that is expressed specifically in satellite cells. In *pax 7^{-/-}* mice, there is a complete absence of satellite cells, however, muscle-derived stem cells are unaffected. These results rather support the idea that satellite cells and muscle-derived stem cells are distinct cell populations. Induction of *pax 7*, therefore, in the muscle-derived stem cells induces satellite cell specification (Seale et al., 2000). Other work has suggested that satellite cells are derived from endothelial precursors (DeAngelis et al., 1999). Bone marrow-derived muscle stem cells have also been shown to contribute nuclei to the regenerating muscle fibers, following intravenous injection into irradiated *mdx* mouse. In the recipient mice, dystrophin expression was restored in 4% of the muscle fibers. This line of research might be beneficial for patients with muscular dystrophy (Ferrari et al., 1998; Gussoni et al., 1999). Skeletal muscle-derived stem cells have also been shown to differentiate to adipocytes, chondrocytes, bone, and smooth muscle (Williams et al., 1999). Other studies have shown that cells residing in the brain or adipose tissue can give rise to muscle (Rietze et al., 2001; Zuk et al., 2001).

In amphibia, however, the skeletal muscle fibers have the ability to re-enter the cell cycle, dedifferentiate, and redifferentiate to form new muscle (Tsonis, 1996; 2000). Such a process is most pronounced during regeneration of the amphibian limbs (see later section).

Heart

The adult mammalian heart is unable to regenerate. As a result, necrosis of heart tissue due to clogged vessels leads to heart failure and death. Also, in other pathological states, such as post-angioplasty intimal hyperplasia, cells of probable smooth muscle origin appear to play a major role. Repairing an affected heart would be of enormous medical interest, because such heart disease is one of the worst killers in humans. Once again, the urodele amphibia have a remarkable capacity of regenerating their hearts after damage or even after tissue removal. The cardiocytes neighboring the affected area can re-enter the cell cycle, they dedifferentiate, and subsequently they redifferentiate to restore the affected part

of the heart (Oberpriller and Oberpriller, 1991). Until recently, it was believed that such a mechanism does not work in mammals and humans. However, evidence has been provided that human cardiac myocytes divide after myocardial infarction (Beltrami et al., 2001). Also in cases where a heart from a female was transplanted in a male recipient, it was found that putative stem cells and progenitor cells exist in the myocardium (Quaini et al., 2002). The MRL mouse also shows remarkable ability for myocardiocyte mitosis after injury (Lefterovich et al., 2001). The search, however, has been recently intensified to identify stem cells that can be used to repair damaged heart tissue. Mesenchymal stem cells have been isolated from bone marrow and injected into the myocardium of recipient animals (Wang et al., 2000). These cells were identified in the host myocardium and had been differentiated, as indicated by the expression of myosin heavy chain and other proteins that are important for contraction. In other studies, stem cell-derived myocytes were shown to occupy nearly 70% of the infarcted portion of the ventricle (Orlic et al., 2001). An alternative method for regenerating affected myocardium is by transplantation of skeletal myoblasts. Such experiments in rabbits showed that when myoblasts were incorporated myocardial performance was improved (Taylor et al., 1998).

Blood vessels

Endothelial cells can be produced either by transdifferentiation or by progenitor cells. Smooth muscle cells and fibroblast have been shown to transdifferentiate to endothelial cells (Beresford, 1999). Endothelial stem cells (ESCs) have been isolated from bone marrow and shown to incorporate in sites of neovascularization. Such transplantation enhanced vascularization in ischemic organs by differentiation and proliferation. Such cells can be useful for applications in regeneration of ischemic tissue (Masuda et al., 2000).

Blood

Blood cells are continuously renewed in the body. This is achieved by the hematopoietic stem cells found in bone marrow. These cells have the ability to produce more stem cells through self-renewal and undergo differentiation to progenitor cells that become the various blood cells, such as white blood cells, red blood cells, T-lymphocytes, mast cells, platelets, dendritic cells and natural killer cells (Orkin, 2001). However, recently it was found that neural stem cells from the brain were able to give rise to different blood cell types including myeloid and lymphoid cells as well as early hematopoietic cells (Bjornson, 1999). This also indicates that some stem cells are not restricted to produce specific cell types, but they show a wider degree of plasticity.

Cartilage and bone

All the components of the skeletal system, bone and cartilage, as well as the connective tissue comprising the tendons and ligaments, are able to repair after injury. During morphogenesis, bone is formed in a particular sequence of events. First, mesenchymal cells proliferate and then differentiate to chondroblasts. This process leads to the production of cartilaginous skeleton. In the following steps, hypertrophy of cartilage is observed with mineralization of the cartilage matrix. The cartilage cells then differentiate to osteoblasts. For this step vascular invasion is necessary. The bone is then remodeled. Such steps are also seen during bone morphogenesis in response to demineralized bone matrix and in fracture healing in adults (Reddi, 2001).

Articular cartilage has limited capacity for a faithful (morphologically and biochemically) repair. Full-thickness cartilage defects are usually replaced by fibrocartilage and partial-thickness defects are repaired by fibrous scar tissue (Ghivizzani et al., 2000). Fibrocartilage formation seems to be mediated by mesenchymal stem cells (MSCs) derived from the bone marrow (Silver and Glasgold, 1995; Prockop, 1997; Pittenger et al., 1999; Jorgensen et al., 2001). These cells can be stimulated to differentiate to chondrocytes in micromass cultures without serum but with transforming growth factor-beta3 (TGF-beta3) (Pittenger et al., 1999). Bone morphogenetic proteins (BMPs) have also been found to stimulate chondrogenesis, and some members of this family have been isolated from cartilage (Reddi, 2001). Also, grafts of perichondrium are successful in repair of full-thickness defects because they contain progenitor cells that give rise to chondrocytes (Silver and Glasgold, 1995).

Bone repair is also mediated by mesenchymal stem cells (Bruder et al., 1994). These cells can be stimulated to differentiate to osteoblasts by culturing them in the presence of serum, dexamethasone, beta-glycerol phosphate, and ascorbate. Also, MSCs can differentiate to osteoblasts by the influence of vitamin D and BMP-2 (Pittenger et al., 1999; Prockop, 1997). Human adipose tissue also contains stromal cells that are able to differentiate to chondrocytes and osteoblasts (Halvorsen et al., 2001; Zuk et al., 2001). Much work has been done on inductive signals for bone morphogenesis, but the emphasis is on BMPs (Reddi, 2000). These proteins have been found to have very specialized patterns of expression during repair of the bone (Bostrom, 1998; Groeneweld and Burger, 2000; Reddi, 2000). During the early stages of fracture healing, some primitive cells express BMPs in the fracture callus. The expression is enhanced in the primitive mesenchymal and chondrocytic cells as the process of endochondral ossification takes place. The expression declines as the cartilaginous component of the callus matures. BMPs are expressed by the osteoblasts, but they decrease as the primitive bone is replaced

by lamellar bone. BMP-2, -3, -4, -5, -7, and -8 are in particular responsible for induction of cartilage and bone formation. BMP-12, -13, and -14 have been derived from cartilage. BMPs have also been used in clinical trials for the treatment of problems with fractures and nonunions (Li and Wozney, 2001; Reddi, 2001).

Tendons, ligaments

It is believed that tendons, ligaments, and other cartilage can be repaired by mesenchymal stem cells as well (Bruder et al., 1994). In general, however, it has been shown that cells that comprise these tissues have the ability for contraction. This has been shown by the expression of alpha-actin, a muscle-specific protein with contractile properties. It has been proposed that such contraction of cells that belong to the above connective tissues might be important for healing and repair of tendons, ligaments, intervertebral discs, and meniscus (Spector, 2001).

Skin

The skin is made up of epidermis and dermis. The epidermis consists mainly of stratified squamous keratinized epithelium, which is capable of continuous renewal. The keratinized epithelial cells are called keratinocytes. Dermis is mostly composed of connective tissue. Renewal of keratinocytes and of the appendages (hair follicles) depends on the proliferation of distinct cell population of stem cells (Ghazizadeh and Taichman, 2001). Despite the controversies in this field, it seems that the stem cells reside in the upper region (bulge) of the outer root sheath (of the vibrissal follicles, present in many mammals). It seems that the bulge contains a population of cells that are able to produce a population of pluripotent and rapidly dividing cells, which subsequently give rise to the hair shaft or to a population of progenitor cells of the epidermis. The presence of multiple stem cells in the cutaneous epithelium with restricted lineages has also been suggested in studies where epithelial cells were labeled with specific genetic markers. However, critics of this idea maintain that single stem cells can give rise to both epithelium and hair follicle (Cotsarelis et al., 1999; Lavker and Sun, 2000; Oshima et al., 2001). Beta-catenin, which is essential in the Wnt/wingless signaling, has been shown to be a paramount factor for stem cell differentiation in the skin. If this protein is mutated during embryogenesis, formation of the placodes that generate hair follicles is inhibited. If beta-catenin is deleted after hair follicle formation, hair is completely lost after the first hair cycle. Also, when beta-catenin is absent, stem cells do not differentiate into follicular keratinocytes but adopt epidermal fate (Huelsen et al., 2001). Other genes involved in impairment of hair fol-

licle development include the Lymphoid Enhancer-binding Factor 1 (LEF-1) and Sonic hedgehog (Shh) (Huelsen et al., 2001). Recent studies have also demonstrated that bone marrow-derived stem cells are able to give rise to skin (Toma et al., 2001; Krause et al., 2001). Another factor that seems to be involved in regeneration of hair and nails is keratin 6 (Wojcik et al., 2000; 2001). Regeneration of sebaceous glands is also attributed to interfollicular epidermal stem cells (Ferraris et al., 1997).

After skin is damaged, however, due to burns or other injuries, regeneration is not very good (however, in first degree and superficial second degree burns, the wound is mainly covered by regeneration tissue). The wound is mainly covered by scar tissue. Research in the area of biopolymers has shown that treatment of such wounds with synthetic membranes composed of a scaffold with extracellular matrix are very promising for enhancing the ability of the skin to heal perfectly (Yannas, 2001). Also, it is interesting to note that human fetal skin heals without scar formation when it is transplanted subcutaneously on an adult athymic mouse, whereas when it is implanted in a cutaneous location, it heals with scar formation. It seems that the fetal fibroblast are the effector cells of scarless fetal skin repair and that TGF-beta might be involved. Presence of TGF-beta 1 results in scar formation, while absence of it results in non-scarring fetal skin (Lorenz et al., 1995; Lin and Adzick, 1996).

Liver

The digestive system in many animals, including humans, has remarkable abilities for regeneration. Among the organs that comprise this system, liver and intestine show great potential for renewal.

There are two models to study liver regeneration in mammals. One way is by partial hepatectomy (PHx) and the other by introducing hepatic toxins, such as CCl₄. After removal of liver lobes, the remaining lobes enlarge to make up for the missing mass. In rats, the whole process takes 5 to 7 days. The body is capable of controlling the right mass. The enlargement of the remaining lobes is achieved through the proliferation of all mature cells that comprise the intact liver. These cells are the hepatocytes (the first to proliferate), the biliary epithelial cells, the fenestrated endothelial cells, the Kupffer cells, and the Ito cells. The ability for liver regeneration has been also verified after repeated hepatectomies. The hepatocytes obviously have an excellent clonogenic capacity. Each rat hepatocyte can expand through 34 cell divisions, and therefore, it can generate 50 livers (Michalopoulos and DeFrances, 1997). At the same time that the hepatocytes proliferate, they are able to perform all vital functions needed for homeostasis. The main factors involved in the induction of hepatocyte proliferation are the Hepatocyte Growth Factor (HGF) and Epidermal

Growth Factor (EGF). They both rise considerably in response to PHx. Other important factors are the Tumor Necrosis Factor (TNF- α), Interleukin-6 (IL-6; secreted for the Kupffer cells and stimulated by TNF- α), and the fifth component of complement (C5). In mice where the genes for TNF- α , IL-6, or C5 were deleted, liver regeneration was largely impaired (Cressman et al., 1996; Yamada et al., 1997; Mastellos et al., 2001). Insulin, norepinephrine, and thyroid hormone are also involved in the mitogenic stimulation of hepatocytes (Michalopoulos and DeFrances, 1997). It is thought that hepatocytes are not terminally differentiated cells and thus they can dedifferentiate, proliferate, and then redifferentiate to mature hepatocytes. In experiments, however, where hepatocyte proliferation was inhibited, it was shown that bone marrow stem cells were the source of hepatic oval cells (Peterson et al., 1999). Hepatic oval cells may be stem cells for hepatocytes and may originate from cells in the canals of Herring or from blastlike cells located by the bile ducts. Local stem cells can also be the source of cells during liver regeneration (Kubota and Reid, 2000; Thiese et al., 2000; Sell, 2001; Vessey and de la Hall, 2001).

Gastrointestinal tract

Cells of the gastrointestinal tract have a rather rapid turnover rate (2–6 days). Stem cells are responsible for the continuous cell renewal in the gastrointestinal tract. These cells reside within the crypts of the small intestine and colon and the mucous cells of the glandular neck of the gastric mucosa. Factors that are involved in such proliferative events include HGF, EGF, TGF, and bFGF. In addition, however, stem cells that home in the gastrointestinal tract have recently been shown to originate from the bone marrow as well (Booth et al., 1999; Jones et al., 1999; Kim et al., 1999; Sattar et al., 1999; Dignass, 2001; Krause et al., 2001).

Pancreas

The pancreas is composed of the endocrine and the exocrine compartment. The endocrine compartment consists of islets of Langerhans, which are clusters of four cell types that synthesize glucagon (alpha cells), insulin (beta cells), somatostatin (delta cells), and pancreatic polypeptide (PP cells). The exocrine compartment consists of the zymogen-containing cells (acinar cells) and the centroacinar cells, ductules, and ducts (ductal tree). During embryogenesis, duct-like protodifferentiated cells appear to be the common originator of all the pancreatic cells. Later in life, the only mitotically active cells are the acinar and the ductal cells, which in fact can transdifferentiate and produce new islet cells (Bouwens, 1998). Factors that may be involved in this

process include members of the EGF, IGF, FGF, TGF, and REG family (Menke et al., 1999; Sumi and Katsuhira, 2000). The ability for pancreas regeneration is good in small as well as in large mammals. Even though bone marrow-derived stem cells have not been reported as a source for pancreas regeneration, it has been shown that mouse embryonic stem (ES) cells are able to differentiate to insulin-secreting structures similar to islets. The cells were initially cultured in the presence of Leukemia Inhibiting Factor (LIF), with subsequent generation of embryoid bodies in the absence of LIF. Then they were selected for expression of nestin. Pancreatic progenitor cells were expanded by treatment of FGF and induction of differentiation to cells secreting insulin was achieved after withdrawal of FGF (Lumelsky et al., 2001). Extension of these studies with human ES cells might be very promising in treating diseases, such as diabetes. In other studies, local stem cells located in pancreatic ducts have been identified as islet stem cells. PDX-1, a major transcriptional factor in pancreas is strongly expressed in the ducts and the progenitor cells (Sharma et al., 1999; Kritzik et al., 1999; Kritzik and Sarvetnick, 2001).

Adrenal gland

The adrenal gland is made up of the cortex and the medulla. The cortex is divided into three zones: the outer zona glomerulosa (zg), the middle zona fasciculata (zf) and the inner zona reticularis. The cortex is covered by the capsule. In mammals, after adrenal enucleation (removal of the cortex), the adrenal cortex is regenerated from the capsule and the adherent cells. These cells begin to differentiate from zona glomerulosa-like to zona fasciculata. The first week after enucleation, there is a preparation for cell division and during the second phase of the first week there is a wave of proliferation. Both glomerulosa and fasciculata cells proliferate in response to enucleation (Taki and Nickerson, 1985; Engeland et al., 1996). Other studies have shown that another layer of cells, found between the zona glomerulosa and zona fasciculata, might be the stem cell zone of the adrenal cortex. These cells express both P450aldo (marker for zg) and P45011 (marker for zf) (Mitani et al., 1995; Engeland et al., 1996). Other experiments have used the method of culturing and transplantation to identify the cell differentiation potential. When cultured zg cells were transplanted into rats after bilateral adrenalectomy, they maintained viability, produced aldosterone and corticosterone, and regenerated a cortex with cells that resemble both zg and zf. On the other hand, transplanted cultured zf cells were not able to regenerate zona glomerulosa and to produce aldosterone. These data suggest that zg cells can acquire the phenotype of zf cells (Tebken and Scheumann, 2000).

Thymus

The thymus consists of lobules, each of which having a peripheral zone of dense cortical lymphoid tissue and a medullary zone. The medulla-cortex compartmentalization is established by the formation of medullary islets, each derived from a single progenitor (Rodewald et al., 2001). The cortical lymphoid tissue is the place where thymocytes or T-lymphocytes are found. The thymus is colonized by lymphocyte-forming cells originating in the bone marrow. In the thymus, undifferentiated cells of bone marrow are induced to become progenitor cells of T-lymphocytes, which are involved in cellular immunity (Heitger et al., 1999). The ability of humans to maintain thymic T-cell regeneration declines with age, and as a result, adult humans regenerate T cells through thymic-independent pathways (Mackall and Gress, 1997a). In thymic-deficient mice, T-cell regeneration occurs mainly by expansion of mature peripheral T-cells, but the animals have limited capacity to restore host immune competence (Mackall and Gress, 1997b).

Thyroid gland

The thyroid gland consists of ball-like structures, the thyroid follicles. Regeneration of thyroid follicles has been observed in disorders, such as subacute thyroiditis. Also thyrocytes can form stable follicles with physiological polarity in 3-D cultures. Such generation of thyroid tissue might be crucial for the treatment of hypothyroidism (Toda et al., 2001).

Lungs

In many mammals, surgical removal of lung lobes leads to rapid compensatory growth of the other lobes. The growth is hyperplastic and not hypertrophic. This rate of growth is maintained until the total lung mass is restored. During growth, the resulting tissue exhibits normal physiological properties and cellular composition (Rannels and Rannels, 1988). The process seems to be affected by hormones, especially adrenal steroids and growth hormones. Pneumonectomy also induces gene expression of immediate-early genes, such as *junB* and *fos* (Gilbert and Rannels, 1998). Retinoic acid has also been found to enhance lung growth after pneumonectomy, and this effect was associated with upregulation of epidermal growth factor receptor (EGFR) (Kaza et al., 2001). Stem cells residing in bone marrow cells have also been shown to differentiate to epithelial cells of the lung (Krause et al., 2001).

Kidney

In humans, mortality from acute renal failure is high, and recovery requires the replacement of tubular epithelial cells to restore tubular integrity (Safirstein, 1999; Hammerman et al., 2000). The growth of the epithelial cells is monitored for a faithful restoration of a single cell layer, so that the tubule architecture is maintained. Several factors have been identified as playing roles in renal regeneration. One of them is HGF (Matsumoto et al., 2000). This factor declines in acute renal failure, and a blocking antibody against HGF leads to acceleration of the disease. Other factors that seem important are EGF and IGF (Hammerman et al., 2000). Also, a gene that is known to be paramount for kidney development, *pax-2*, is expressed in the tubular epithelial cells in mice injected with an agent that induces experimental acute tubular necrosis. *Pax-2* expression seems to recapitulate the developmental pattern in order to restore the mature kidney (Torres et al., 1995; Imgrund et al., 1999). Other diseases, such as renal organ deficiency and chronic renal failure are serious, but not many studies on the regeneration front have been established.

Germ cells

Germ cells in the female and male reproductive system give rise to gametes and they are produced by the primordial germ cells that migrate during early embryogenesis to the gonads. The differentiation of germ cells to form mature eggs or sperms is a very complicated process controlled by many hormones and factors. During spermatogenesis, germ cells (early spermatogonia) divide and differentiate to spermatocytes, which then enter meiosis to produce the spermatids and finally the spermatozoa. This process is continuous. In the adult, germ cells are believed to arise from stem cells or progenitor cells. Since these stem cells are only destined to produce germ cells that will differentiate only to gametes, it might be safer to call them progenitor cells because their differentiation potential is limited. In the female reproductive system, the germ cells are limited in numbers, they differentiate to a variety of cells before they become mature eggs, but continuous renewal is not the case (Hogan, 2001).

The amazing case of amphibian limb regeneration

The review so far has mainly dealt with the ability for regeneration in mammals and the importance of local or bone marrow-derived stem cells for tissue renewal. Regenerative abilities of lower animals, however, are far more impressive. We all know that some invertebrates are able to regenerate their bodies even if they are cut in

half! Such regeneration is thought to be the result of local cells, which are produced by somatic cells by dedifferentiation. Stem cells do not seem to play much of a role in these processes. Of course there are exceptions to this. Planaria regeneration is thought to be mediated via neoblasts, which are pluripotent stem cells. Alas, such remarkable regenerative abilities in vertebrates belong only to the realm of science fiction. The only exception, where we see remarkable regenerative abilities in lower vertebrates, is in some salamanders. The regeneration of lens and retina in these animals was described in previous sections, and other abilities of these animals (brain, spinal cord, heart) were mentioned. Among all the parts that are able to regenerate in salamanders, perhaps regeneration of the limbs is the most spectacular (Tsonis, 1996; 2000). Upon amputation of the limbs and following the closure of the wound by a specialized epithelium, the cells of the stump (already terminally differentiated muscle, bone, cartilage and other tissues) dedifferentiate and produce a population of cells, called the blastema. The process is most dramatic for the muscle fibers, which virtually melt down to mononucleated cells. Such a re-entry to the cell cycle is not possible in mammalian myotubes, unless they are transfected with *msx-1* (Odelberg et al., 2000). The blastema undergoes a period of intense proliferation and then redifferentiates and produces an exact replica of the lost part of the limb. The regenerated limb is produced by means of transdifferentiation of the intact cells. It has been documented that muscle-derived blastema cells can become chondrocytes during regeneration (Tsonis, 1996; Lo et al., 1993). The process of transdifferentiation has been shown by elegant experiments where labeled mononucleated muscle cells are found in fused myotubes in the regenerating limbs (Lo et al., 1993). The role of muscle satellite cells or stem cells in limb regeneration remains largely unknown. Recent work is concentrating on identifying the factors that might control such a remarkable event. Retinoblastoma (Rb) protein seems to be specifically phosphorylated during the cell cycle re-entry and this is stimulated by thrombin (Tanaka et al., 1997; 1999). This might be an early signal for dedifferentiation. Other factors that seem to correlate with the ability for limb regeneration in amphibia are FGFs and their receptors (D'Jamoos et al., 1998; Yokoyama et al., 2001).

Synthesis and future prospective

This review has presented a synthesis dealing with the regenerative capability of the different tissues and organs in vertebrates. I believe that this synthesis clearly illustrates that the emergence of the new field of Regenerative Medicine is one of the best examples in the history of Life Science of how very basic biological research provided the foundation of what it could be a medical

revolution. The reader should be left at this point with a good appreciation of the huge potential for regeneration of the different body organs. While the regenerative potential weakens in more advanced vertebrates and mammals, it is obvious that the capacity for regeneration can be induced with the appropriate stimulus. Two main avenues provide the vertebrate body with regenerative potential. One is by transdifferentiation of local terminally differentiated cells. This is clearly very spectacular and is prominent in the regenerative processes in lower vertebrates, such as amphibia, but it can also be seen during organ regeneration in mammals, including pancreas and adrenal gland. Terminally differentiated cells involved in such mode of regeneration must respond to specific signals in order to re-enter the cell cycle and change their genetic program to enable them to change their phenotype. The second avenue whereby renewal can be achieved is by stem cells, which can be local or derived from other tissues (mostly from the bone marrow; see Table 2). The stem cells could be totipotent (fertilized ovum or cells before the formation of the blastocyst), pluripotent (capable of differentiating to many lineages, depending on the signals), or having the ability for differentiation to particular cell lineage (progenitor cells). The existence of such cells in the body is of great promise for their use in tissue renewal. The existence of ES cells is of particular importance. These cells have been shown to differentiate to many cell types, and theoretically they can be pluripotent (Smith, 2001; Wakayama et al., 2001). Identification of stem cells and

Table 2 Plasticity of stem cells

Stem cells	
Reside	Can become
brain	neurons, astrocytes, oligodendrites
brain	skeletal muscle
bone marrow	neuronal cells
skeletal muscle	skeletal muscle
skeletal muscle	fat, bone cartilage, smooth muscle
bone marrow	skeletal muscle
bone marrow	cardiac muscle
myocardium	myocytes, endothelial cells
bone marrow	endothelial cells
bone marrow	blood cells
brain	blood cells
bone marrow	cartilage, bone, adipocytes
skin	keratinocytes, skin appendages
bone marrow	skin
bone marrow	oval cells (liver)
liver	liver cells
intestine, colon, gastric mucosa	cells of gastrointestinal tract
bone marrow	cells of gastrointestinal tract
bone marrow	thymus
testis, ovaries	gonads
bone marrow	epithelial cells of the lung
pancreatic ducts	islet cells
adipose tissue	bone, cartilage, muscle, fat

of the signals that channel their differentiation to different cell types could, in principle, provide the means to re-populate damaged tissues with healthy cells. It is my conviction, however, that both avenues should be pursued rigorously in the field of Regenerative Medicine. It could be possible that a local cell that dedifferentiates and then differentiates to a particular cell type (say chondrocyte) needs the same signal as a mesenchymal stem cell differentiating to chondrocyte. In other words, these two cells could be of the same genotype. Also, the ability of a somatic cell to transdifferentiate draws a parallel with the ability of a stem cell to differentiate to a particular progenitor cell. In this sense, studying the two different avenues that are used in regeneration should complement each other. Research should be pursued to show the common aspects of the different mechanisms involved in regeneration. Another area of research that should be incorporated in the study of Regenerative Biology and Medicine is that of bioengineering. Advantages in this field have provided the means used in healing processes and should complement biological and medical experiments. The unity of basic biologists, medical researchers, and bioengineers is destined to create one of the most exciting branches in medicine for the years and centuries to come.

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