Regeneration of skeletal and cardiac muscle in mammals: do nonprimate models resemble human pathology?

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Most of the available information regarding the regenerative potential and compensatory remodeling of mammalian tissues has been obtained from nonprimate animals, mainly rodent experimental models. The increasing use of transgenic mice for studies of the mechanisms controlling organogenesis and regeneration also requires a clear understanding of their applicability as experimental models for studies of similar processes in humans and other mammals. Application of modern cell biology methods to studies of regenerative processes has provided new insights into similarity and differences in cellular responses to injury in the tissues of different mammalian species. During more than 200-million years of progressive divergent evolution of mammals, cellular mechanisms of tissue regeneration and compensatory remodeling evolved together with increasingly adaptive functional specialization and structural complexity of mammalian tissues and organs. Rodents represent a phylogenetically ancient order of mammals that has conservatively retained a number of morphofunctional characteristics of early representatives of this class, which include enhanced regenerative capacity of tissues. A comparative analysis of regenerative processes in skeletal and cardiac muscle, as well as in several other mammalian tissues, shows that time courses and intensities of regeneration in response to the same type of injury vary even within taxonomically related species (e.g., rat, mouse, and hamster). The warm bloodedness of mammals facilitated the development of more complex mechanisms of metabolic, immune, and neurohumoral regulation, which resulted in a stronger dependence of regenerative processes on vascularization and innervation. For this reason, interspecies modifications of regenerative responses are limited by the capacity of the animal to resorb rapidly the foci of necrosis and to revascularize and reinnervate the volume of the regenerating tissue. These differences, among other factors, result in significantly lower rates of reparative regeneration in mammals possessing larger body sizes than rodents. A review of these data strongly indicates that the phylogenetic age and biological differences between different species should be taken into account before extrapolation of regenerative properties of nonprimate tissues on the regenerative responses in the primates. **(WOUND REP REG 1999;7:26–35)**

The adequacy of modeling in biomedical research still remains one of the most controversial and ill-understood problems of modern life sciences. The paraphrase "to muddle or to model, that is the question" was used as the epigraph to one of the recent reviews concerning the principles and philosophy of modeling in present-day experimental biology and medicine.[1] The applicability of rodents and other nonprimate animals as models in cardiovascular physiology and aging research has been debated in a number of publications.[2–5] Significant dissimilarities between different taxonomic groups of mammals also exist at the cellular level. For instance, rodent and human cells differ in responses to carcinogens in vivo and in cell culture.[6–9] Mouse cells of different tissue origin possess significantly higher capacity for spontaneous immortalization and induced transformation in vitro than the cells of many other species. Cell lines isolated from normal and malignant mouse tissues better retain some differentiative properties in vitro. This explains why many permanent cell lines are of mouse

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origin and why the vast hybridoma industry is based on the use of mouse cells. Other growing areas of application of rodent models include studies of mechanisms controlling normal and abnormal tissue growth and differentiation in transgenic mice.

Widespread application of rodent models to study the mechanisms controlling differential gene expression during normal development and under conditions of pathology inevitably raises questions regarding the extent of similarity and differences in the regulatory pathways in rodents and humans. Noteworthy, most of the data concerning the regenerative properties of mammalian tissues have been collected in rodent models. However, what still remains unclear is the degree of reliable homology between the regenerative responses observed in mammals belonging to different systematic groups.

One of the paradigms of modern research mentality inspired and perpetuated by the molecular biological revolution is the tendency to generalize and extrapolate the conclusions of experiments with rodent models to common properties of all mammalian species. To some extent, this assumption has evolved from the radical reductionism of the 1960s, with its belief in conserved evolutionary rigidity of major regulatory pathways in vertebrate species at the cellular level. This approach led to the tendency of reducing the problem of tissue regeneration and remodeling to activation of cellular proliferation in resting differentiated cells surrounding the zone of injury. Identification of the genes controlling proliferation, differentiation, and morphogenesis in different tissues during normal histogenesis and attempts to activate the regenerative process by induced expression of these genes became the dominant trend in modern regeneration research. The development of novel therapeutic approaches such as gene transfer, growth factor therapy, and direct transfer of proliferating cells is also aimed at the activation of mitotic activity at the site of injury. Application of these approaches to skeletal and cardiac muscle regeneration, as well as general aspects and cellular mechanisms of regenerative processes in these tissues, has been discussed in recent reviews.[10–15]

Although it is evident that activation of the proliferative response is one of the key events necessary for successful tissue repair, this is definitely only one side of this complex multifaceted problem. It is apparent that the factors underlying the activation, intensity, and completeness of regeneration are not limited to intrinsic proliferative and differentiative properties of cells in the injured tissue. A number of integrative organismic determinants diverging in different systematic groups of mammals can affect the process of tissue regeneration. A convincing illustration of this influence is strong dependence of the regenerative process in skeletal muscle on innervation and revascularization.[12,15]

United under the term *striated muscle*, skeletal musculature and the myocardium of the Vertebrata differ profoundly in pathways of phylogenetic development, histogenesis, and regeneration, despite the close structural similarity of their sarcomeric contractile system. The purpose of this article is to analyze and explain the origin of the differences in regenerative and compensatory processes observed in mammalian skeletal and cardiac muscle in terms of the systematic position of the animal. Elucidation of these differences is important for clearer understanding of applicability of data obtained in studies of animal models to regenerative processes in the human tissues.

COMPARATIVE ASPECTS OF REGENERATION IN MAMMALS: STEREOTYPES AND REALITIES

The regenerative capacity of mammalian tissues and organs is generally considered to be significantly lower than that of amphibia and reptiles. However, this is not quite true. For example, the magnitude and intensity of reparative regeneration in rat liver significantly exceed the responses to injury observed in frog and newt liver.[16–18] Similar differences have been found between mammalian and amphibian skeletal muscle. In skeletal muscle regenerating after mincing, the first multinucleated myotubes were observed by day 3 following injury in the rat, day 8 in the frog, and only by days 9–10 in the axolotl.[19] Reactivation of DNA synthesis and mitotic activity in the atria of the rat heart is more rapid and intense than in the atria and ventricles of the newt heart.[14,20–22] These examples demonstrate the capacity of at least several types of mammalian tissues to reactivate proliferation in response to injury more rapidly and intensely than what occurs in injured amphibian tissues.

The remarkable capacity of rat liver to regenerate 75%–90% of its mass after resection and even after repeated hepatectomies has made this experimental model a classic object for studies of early and advanced stages of tissue regeneration in mammals.[16,23] However, unlike rat liver, human liver regenerates much more slowly and less completely even after more moderate partial resections.[24] Early studies showed that liver regeneration is better in small animals with a rather short life span.[25] Rat liver usually completes its

regenerative process by days 8–16 following major hepatectomy,[16,23] whereas in humans, the similar process requires more than 3–4 months.[24]

Another example of the great regenerative potential of mammalian organs is regeneration in the rat pancreas. Resection of up to 80%–90% of the pancreatic mass is followed by an intense regenerative response.[26–28] Similar regenerative reactions occur after chemical destruction of liver with carbon tetrachloride[16,23] and the pancreas with ethionine.[26,29] These later studies indicate that mechanical trauma and the loss of the tissue mass per se are not necessary for reactivation of cell proliferation. Destruction of 80%–90% of the pancreatic acinar cells by ethionine in the rat is followed by a return to histological normality and normal weight within 28 days.[29] Again, in humans, the process is considerably slower and less intense. Numerous complications of pancreatectomy and moderate success in transplantation of insulin-producing cells have been described in clinical practice.[30] Taken together, these examples illustrate a high regenerative capacity of selected mammalian organs, as shown in experiments with rodent animal models, and they demonstrate the differences between rodents and humans in the dynamics of the regenerative response.

To understand better the nature of the different regenerative capacities of mammals, we briefly consider the phylogenetic history of the classes of mammals. The first mammals appeared approximately 220- to 210-million years ago, at the beginning of the Jurassic period of the Mesozoic era.[31] Because of their great adaptive potential, they overlived many extinct taxonomic groups, such as terrestrial and marine giant reptiles, many species of cephalopod mollusks, as well as most primitive birds that evolved from reptiles after the appearance of early mammals on the evolutionary scene. The first mammals were small shrew-like animals, and today, nearly half the 4,000 living mammalian species are diminutive rodents.[32] For comparison, the order of primates is comprised of only approximately 150 living species.

Diverging selection pressures during the course of evolution resulted in the formation of mammalian species possessing large body sizes. Such animals proved less competitive and more vulnerable to changing environment, and most of these species are now extinct. An example of this trend is the extinction of many representatives of abundant mammalian fauna that throve during the Oligocene and Pleistocene epochs, including all species of mastodons and saber-toothed cats. It is interesting that the tendency toward predominant extinction of animals possessing large body sizes has been observed in all classes of vertebrates and invertebrates. In this respect, small sizes of a great part of living fishes, amphibia, reptiles, and birds also illustrate this trend. Thus, because of their perfect adaptation to specific habitats, the phylogenetic ancestors of modern rodents gave rise to a large branch of species that evolved in parallel to a major vector of progressive evolution of phylogenetically younger mammals.

At this point, it should be mentioned that the evolution of mammalian species, as an adaptogenic process, resulted in the development of a wide variety of profound morphofunctional modifications at the body and organ levels. A convincing illustration of this process is divergent evolution of terrestrial and sea mammals, which apparently occurred under similar selection pressures as the earlier bifurcation of the evolutionary tree of the terrestrial and extinct marine reptiles. Another product of this divergence is a wide diversity of life spans, lifestyles, longevity, and reproductive cycles of modern mammals. Thus, changing selection pressures led to the progressive ecological specialization of mammals, which resulted in differences in diets, bipedal or tetrapedal types of locomotion, and speed of movement. Such a specialization required corresponding adaptations of the locomotive and cardiovascular systems and was accompanied by morphofunctional modifications of skeletal and cardiac muscle.

Interestingly enough, the evolutionary changes were not limited to modifications at the level of systems and organs, such as progressive development of the central and peripheral nervous systems, vascular/humoral regulation, evolution of the skull and the whole skeleton, behavioral patterns, etc. Significant adaptive and compensatory modifications also occurred at the cellular level and involved characteristics of terminal differentiation and responses to hyperfunction in skeletal and cardiac muscle cells of different mammalian species. Later here, we discuss how these species-specific modifications at the systemic and cellular levels directly correlate with the regenerative and compensatory responses of mammalian cardiac and skeletal muscle.

REGENERATIVE RESPONSES IN SKELETAL MUSCLE OF DIFFERENT MAMMALIAN SPECIES

For a long time, the skeletal muscle of mammals has been considered to possess low regenerative ca-

Table 1. Regeneration of skeletal muscle in different species of mammals

Animal	Type of injury	Intensity of regeneration	Testing technique	Reference
Rat	Mincing and grafting	++++	HS, AR	15, 34, 39
	Marcaine damage	++++	HS, EM	15, 47, 48
Mouse	Mincing and grafting	++++	HS, AR, IC	83, 84
	Crush injury	++++	HS, AR, IC	40, 41
Guinea pig	Mincing and grafting	+	HS	34
Rabbit	Mincing and grafting	++	HS, EM	34
Cat	Mincing and grafting	+++	HS, EM	38, 39
Dog	Mincing and grafting	+ or +++	HS	34–37
Human	Mincing and grafting	-	HS	39

Intensity and quality of regeneration: ++++ very good; +++ good; ++ fair; + poor; - no regeneration. Testing techniques: HS, histology; AR, 3H-thymidline autoradiography; EM, electron microscopy; IC, immunocytochemistry.

pacity. Hudgson and Field describe the history of early studies on skeletal muscle regeneration.[33] One of the first pieces of evidence of a considerable difference in the regenerative properties of skeletal muscle between mammalian species was obtained by Studitsky et al.[34] These authors observed an intense regenerative process and restoration of the contractile function of muscle after the harsh injury of mincing the muscle into small pieces and autografting of this minced muscle into the site of its normal anatomical location.[34] These experiments revealed considerable interspecific differences in the regenerative capacity of skeletal muscle subjected to the same type of injury. The regenerative process was the most effective and successful in rats and was the weakest in dogs. It is interesting that the muscle tissue of other species of rodents, such as rabbits and guinea pigs, regenerated much less intensely and less completely than the rat tissue. Later, two laboratories attempted to perform grafting in dogs and, similar to Studitsky, failed to obtain successful regeneration,[35,36] but another group claimed more positive results.[37] Studitsky attributed the rapid and effective regenerative response in rat muscle to the high capacity of this animal to resorb the necrotic tissue in the zone of injury and to activate the inflammatory reaction. It was pointed out that the number of polymorphonuclear leukocytes and macrophages participating in inflammatory response and clearing the necrotic tissue was significantly higher in rats than in blood of other studied mammals.[34] In later studies of regenerating muscle grafts, Carlson and Faulkner expanded the number of studied species to cats and monkeys.[38,39] Although in cats the regenerative process included similar stages, it was significantly prolonged. According to the authors, "What takes one day in the rat, takes one week in the cat."[39] Some degree of regeneration was also observed in monkeys, whereas incidental attempts to apply this technique in the clinic for restoration of injured large human muscles (such as the tibialis anterior) resulted in the substitution of degenerating muscle by connective tissue.[39] Recent studies of McGeachie and Grounds [40–43] demonstrated differences in the time course of regeneration between different strains of laboratory mice. Further development and modification of the techniques of skeletal muscle transplantation, such as nerve-intact free grafting and microsurgical repair of nerves and blood vessels, permitted the introduction of muscle transplantation into clinical practice.[44,45] The examples of different regenerative responses of skeletal muscle to similar types of injury in mammals belonging to different taxonomic groups are presented in Table 1.

The total mass of the regenerating tissue is one of the most important determinants of successful regeneration. Faulkner et al. concluded that the upper limit of tissue mass that permits muscle regeneration after grafting is approximately 6 g.[46] The authors speculated that failure of larger grafts to regenerate arose from difficulties with revascularization and reinnervation of the regenerating transplant. This explains why muscle grafts recover significantly better in small animals than in mammals possessing large body sizes. Another important factor that may hamper regeneration is overgrowth of the regenerating muscle with connective tissue. Proliferation of fibroblasts and accumulation of collagen deposits create a mechanical obstacle for fusion of myoblasts and migration of macrophages and also prevent revascularization and reinnervation of the regenerating tissue. The importance of complete and fast resorption of atrophying and degenerating muscle fibers for effective regeneration was demonstrated in experiments with Marcaine-induced damage of the muscle. This myotoxic local anesthetic induces rapid degeneration and breakdown of

mature muscle fibers without affecting the survival of myogenic satellite cells.[47–49] It was shown that injection of Marcaine into grafted muscle or soaking muscle tissue in Marcaine solution before grafting result in faster and more complete regeneration. Despite significant acceleration of the regenerative process, the morphological and functional restoration of muscle tissue is usually more complete with Marcaine treatment than after regeneration without Marcaine treatment. It is interesting that the stimulating effect of this drug on regeneration can be enhanced if hyaluronidase is added to the Marcaine solution. Under these conditions, the regenerated muscle contained smaller amounts of the connective tissue and better developed and organized muscle fibers.[48] This indicates that the cleavage of some tissue components containing chondroitin can activate and facilitate the regenerative process.

The importance of neurohumoral trophic support for muscle regeneration has been elegantly shown by Carlson and Faulkner in experiments with cross-age transplantation of muscle grafts in rats.[50] It was shown that the transplanted grafts of old tissue regenerated very well in young hosts, which indicated that the age-related impairment of regenerative capacity can be accounted for, at least partially, by changes in the factors of neurohumoral trophic environment. These authors also showed that the force deficit in regenerating grafts results not from a failure to regenerate, but a failure to reinnervate a normal number of muscle fibers.[51]

Similar experiments with cross-transplantation of muscle grafts between different strains of mice[43] showed that the difference in the time course of regeneration were underlain by systemic organismic factors rather than the intrinsic properties of myogenic cells. However, some properties of myogenic satellite cells do differ between mammalian species. Allen et al. found that bovine and rat myogenic cells in culture differed morphologically and exhibited different patterns of expression of the muscle-specific intermediate filament desmin.[52] Unlike myosatellites of human muscle, mouse myogenic cells isolated in culture can give rise to permanent cell lines after appropriate propagation and selection.[53] The terminally differentiated phenotype of mature muscle fibers also varies in different groups of mammals. For example, in humans, most muscles are of mixed types,[54] whereas in rodents, specialization into fast and slow types is more strictly differentiated.[55]

The role of innervation as an important trophic factor modulating the regenerative process of skeletal muscle is well established.[15] However, regenerating muscles of different species show different sensitivities to denervation. In frog and mouse, the nascent muscle fibers undergo complete degeneration during the first 3 weeks of aneural regeneration.[56,57] Rat muscles do not exhibit such dependence on the presence of the nerve, and the process of atrophy in the regenerates is significantly slower.[58,59] Responses of intact adult muscles to acute denervation also revealed species-specific differences. Lewis et al.[60] concluded that the responses to denervation of soleus muscle of rats differ profoundly from those in some other mammalian species. Denervation results in much more pronounced atrophy in rat soleus compared with the same muscle in guinea pigs and cats.[60] Another type of pathology that may differ between rodents and humans is age-related muscle atrophy. Humans lose approximately 50% of muscle mass between the ages of 25 and 75 years,[61] whereas in rodents, the age-related loss of muscle mass appears to be less dramatic.[5] Taking into account the evidence indicating that age-related muscle atrophy is underlain by the loss of innervation,[62] the difference between humans and rodents may be explained by a higher capacity of rat tissue for the compensatory remodeling of motor units. It is interesting to note that during both denervation-induced atrophy and aging, the compensatory regeneration of muscle does not occur despite the presence of a large reserve of myogenic satellite cells.

Thus, the comparative analysis of the regenerative and compensatory responses of skeletal muscle reveals significant differences in the regenerative properties between mammalian species.

REGENERATIVE RESPONSES IN CARDIAC MUSCLE OF DIFFERENT MAMMALIAN SPECIES

The hearts of mammals differ not only in weight, size, and shape but also in growth patterns and characteristics of terminal differentiation of cardiac muscle cells. Unlike nonprimate mammals, the ratio of heart weight to body weight in humans progressively increases from neonatal stages to adulthood.[63] Although the functional significance of this difference still remains unclear, one can hypothesize that this physiological peculiarity may be explained by a higher hemodynamic load and functional demand resulting from the necessity to pump the blood vertically in the erect body.

Cardiac muscles of mammals also differ at the cellular level. In the hearts of rats and mice, the greater

part of muscle cells (80%–90%) in ventricles contains two diploid nuclei. Up to 1%–2% of myocytes have four nuclei, and the rest of the cell population is mononucleated.[64] In the pig heart, the number of multinucleated myocytes containing more than two diploid nuclei comprises on the average 50%, and approximately 15% of myocytes are multinucleated.[64,65] In the human heart, up to 45%–70% of the muscle cell are binucleated.[64,66] Multinucleation of muscle cells is especially typical of the pig heart, where rows consisting of 6–20 myonuclei can be observed.[65] Thus, in the hearts of rodents and many other mammals, true nuclear polyploidy is a rare event. Unlike most nonprimate mammals, nuclei in cardiac myocytes of humans are predominantly polyploid with the modal DNA content within the 4C and, to a lesser extent, 8C classes[20,66,67] Similar to the human heart, the pig heart possesses 15%–55% of myocytes with 4C nuclear DNA content and a relatively large number of cells with ploidies greater than 4C.[65] It is interesting, for comparison, that in the liver, significant levels of polyploidy were found only in hepatocytes of 5 out of 30 species studied.[64] These data show that during terminal differentiation in all studied species of mammals, muscle cells in the heart acquire more or less significant levels of genome multiplication. This is attained both as development of real nuclear polyploidization, such as in primates, or as a result of repeated karyokineses causing an increase of the number of nuclei, such as in nonprimate species. Considerably higher levels of polyploidy in the human myocardium appear to be associated with a large body size and the bipedal type of locomotion of *Homo sapiens*. Polyploid myocytes containing a multiplied number of genes encoding contractile proteins may prove more effective for generation of mechanical force and transportation of the blood vertically in a large body against gravitational forces. The development of similar cellular mechanism in pigs may be explained by a relatively small size of the heart that should effectively serve the needs of a very heavy body. Another important adaptive characteristic of human cardiac muscle is its capacity to reactivate DNA synthesis under conditions of functional overload and hypertrophy. Significant increases in nuclear ploidy levels were observed in hypertrophied human hearts subjected to protracted functional insufficiency and overload.[20,67,68] This response of the human heart to hyperfunction is different from that observed in the rodent heart. No significant reactivation of DNA synthesis was observed in different models of cardiac hyperfunction in rats and mice.[69–71] The responses of the human and nonprimate hearts to overload also differ at the level of the whole organ. Unlike several nonprimate species, the human heart considerably increases its weight under conditions of protracted hyperfunction. In rodent models of cardiac overload, the weight of the heart increases by 20%–80%, whereas the human heart can double and triple its normal weight.[20,67,70,72]

Until recently, cardiac myocytes of mammals were considered to be terminally differentiated cells unable to re-enter the mitotic cycle.[73] Presently, the rat heart still remains the most widely used model in basic heart research. However, the pathogenesis and response to injury in the rat myocardium have species-specific features. For example, unlike the hearts of many large mammals, the rat heart has a poorly developed system of collateral blood flow, and this explains why myocardial infarction in these animals is frequently transmural.[74] During the course of evolution, phylogenetically younger mammalian species developed collateral circulation as an important compensatory mechanism that provides better adaptability, stability, and protection of the heart function after injuries and during overload. This illustrates the general evolutionary trend toward progressive functional specialization of the organs observed in vertebrate species. To this end, it is interesting that the amphibian myocardium is practically avascular and does not have intramural blood vessels. Its spongelike trabeculated structure allows free circulation of the blood inside the ventricular wall.[21] The rate of heart contractions, oxygen consumption, and metabolic rate in the myocardium of warm-blooded animals is considerably higher than those in amphibian and reptile hearts, which explains the functional necessity of myocardial vascularization in mammals. Interestingly enough, in the embryonic hearts of birds and mammals, the avascular trabeculated heart is a transient stage of development that recapitulates the final pattern of organogenesis in their phylogenetic ancestors.

The response of the heart to injury and overload varies even between individual species belonging to the order of rodents. For example, the proliferative response to myocardial injury induced under the same experimental conditions following isoproterenol injection is higher in mice than in rats.[75] The presence of a very small number of DNA-synthesizing myocytes and rare mitotic muscle cells in the perinecrotic zone was reported in several mammalian species, including humans, after experimental myocardial injuries of different etiology.[20,73] This very modest proliferative response localized in the area surrounding the site of

Table 2. Regeration of cardiac muscle in different species of mammals

Animal	Type of injury	Manifestations of regeneration in the perinecrotic zone	Testing technique	Reference
Rat	Infarction	S,M	HS, EM, AR, FC, IC	20, 85, 86
	Isoproterenol injury	S	HS, AR, IC	20, 75, 87
Mouse	Isoproterenol injury	S	HS, AR	20, 75
Rabbit	Infarction	S, M	HS, AR	20
Human	Infarction	S, M	HS, QF	20, 75, 88

Manifestations of regeneration: M, mitotic myocytes; S, DNA synthesizing myocytes. Testing techniques: HS, histology; AR, autoradiography; EM electron microscopy; FC, flow cytometry; MI, direct counts of mitotic cells; OF, quantitative DNA photometry.

injury is not sufficient to provide significant regeneration of cardiac muscle. Rapid activation of fibroblast proliferation during the first days after trauma and activation of collagen synthesis result in formation of a connective tissue scar at the site of injury. The data concerning the proliferative reactivity of cardiac muscle cells in the perinecrotic zone of the injured heart in different species of mammals are summarized in Table 2. Taking into account the increasing controversy of these data, it appears possible that some of the recent reports overestimate the real number of proliferating cells in the injured and overloaded mammalian heart. It should be noted that, similar to skeletal muscle, resorption of the necrotic cells at early stages of tissue repair and formation of the scar are faster in the rat heart than in the hearts of several studied mammalian species, including humans.[76–79]

Taken together, these results indicate that the pathways of terminal differentiation and responses of cardiac muscle to injury and hyperfunction depend on the systematic position of the animal.

SUMMARY

Even a brief review of data concerning regeneration of skeletal and cardiac muscle in mammals clearly shows that the processes of morphological and functional restoration of these tissues have distinct species-specific features. During more than 200-million years of divergent evolution, thousands of mammalian species emerged, evolved, and vanished in a wide variety of diversified terrestrial and marine habitats. The phylogenetic development of mammals resulted in both quantitative and qualitative evolutionary changes in the functional properties of their organs and tissues. An illustration of such a remarkable evolution is the progressive increase in brain size and its morphofunctional complexity that led to the development of intelligence in the most evolutionarily advanced order of mammals, the primates. A similar example of progressive functional specialization is the development of the polyploid heart and advanced level of collateral circulation. This process of progressive adaptive evolution affected the organization and properties of many other tissues and organs in vertebrate and invertebrate animals.

A comparative analysis of the regenerative capacities of tissues and organs in different species of mammals clearly indicates that the phylogenetic age of a species is one of the factors determining its regenerative properties. To this end, the enhanced regenerative potential exhibited by rat tissues might be explained by evolutionarily conserved retention of some characteristics inherited from the early mammals. The early mammals evolved from reptile ancestors and probably possessed a higher regenerative capacity typical of reptiles. However, it is becoming increasingly apparent that some cellular mechanisms necessary for rapid and effective tissue repair have been developed during the evolutionary "arms race." Such adaptations were vital for the survival and better fitness of species under conditions of their habitats. For instance, the capacity for rapid and effective resorption of the necrotic cells and phagocytosis at early stages of tissue repair in some rodents was acquired during the course of evolution of their immune system. Such an adaptation provided higher resistance of rodents to infections and different kinds of injuries and therefore improved their competitiveness in natural selection. Enhanced regenerative capacity also provided some selection advantages to rodents as frequent prey to hunting efforts of reptiles, birds, and carnivorous mammals. Thus, diverging selection pressures in ecologically different habitats lead to evolutional modifications of regenerative reactions. At the same time, independent evolution of isolated populations with a more or less significant degree of inbreeding can result in differences in the dynamics of regenerative response, as was described

in several strains of laboratory mice. In the wild, a similar type of microevolution occurs during the course of population development on isolated islands.

It should be noted that the concept of parallel, independent evolution of functionally homologous tissues in different systematic groups of animals was hypothesized on the basis of comparative morphofunctional studies during the era of classic histology.[80] The modern data concerning evolutionary modifications of the patterns of histogenesis and regeneration provide new evidence supporting this principle of evolutionary dynamics of tissue development and repair.

Several important issues should be taken into account when we consider future prospects of regeneration research in mammals and clinical applications of such novel techniques as growth factor therapy and cell-transplantation therapy. Complete regeneration assumes full morphological and structural restoration of the tissue. To attain this goal, the regenerating tissue should sustain coordinated growth, differentiation, and spatial interrelations of stromal versus parenchy cells and develop normal patterns of innervation and vascularization. The time span for completion of regeneration depends on the proliferative and morphogenetic potential of several cell types, and each of these types may have different kinetics of proliferation and differentiation in response to the same type of mitogenic stimulus. Studies of cultured fibroblasts isolated from animals belonging to 10 different species have revealed a correlation between the number of population doublings in vitro and the maximum life span of the animal.[81] It still remains unclear what is the real proliferative potential of myogenic satellite cells in different species and what is the contribution of the progeny of single myoblasts to the formation of new regenerating muscle fibers. Experiments with clonal cultures indicate that mouse and human myoblasts can give rise to colonies consisting of hundreds of cells.[53] A recent report concerning the stem-cell–like proliferative potential of adult mouse hepatocytes and their capacity to repopulate large areas of damaged liver with a progeny of single cells[82] also indicates that the replication capacity may differ in different cell types. However, no data are presently available concerning direct side-by-side comparison of the proliferative behavior and differentiative properties of human and animal cells of different tissue origin. New information identifying these tissue-specific and species-specific differences will bring better understanding of applicability of the data obtained in studies of experimental animal models to clinical setting.

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