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Throughout the human life cycle, tissues are regenerated either continuously to maintain tissue integrity in the face of normal cell turnover or in response to acute or chronic damage due to trauma or disease states. Blood, epithelia of skin and tubular organs, hair and nails, and bone marrow are examples of human tissues which regenerate continuously as well as in response to damage. Bone,[1] muscle,[2] adrenal cortex[3] and kidney epithelium[4] also regenerate in response to damage, and bone is continually remodeled in response to stress vectors.

The response of many other vital tissues to damage, however, is not regeneration but repair by the formation of collagenous scar tissue.[5] Scar tissue interrupts the normal tissue architecture, compromising its function to varying degrees, depending on the severity of the injury or disease process. Some examples of diseases and injuries that cause serious impairment by scarring are third degree burns, spinal cord injuries, diabetes, macular degeneration of the neural retina and myocardial infarction. These injuries and diseases are costly in terms of healthcare dollars and potential decline in quality of life. Thus, a major goal of biomedical science is to be able to restore the structure and function of damaged or dysfunctional tissues that do not regenerate naturally. Three major approaches to tissue restoration have been developed: bionics, solid organ transplantation, and, more recently, regenerative biology, which includes cell transplantation, bioartificial tissue constructs and regrowth of new tissues from injured residual tissues in vivo. The purpose of this paper is to discuss these approaches with a particular emphasis on regenerative biology.

BIONICS AND SOLID ORGAN TRANSPLANTATION

Over the past 50 years, we have made remarkable advances in the use of bionic devices and solid organ transplants as replacement parts for failing tissues and organs.

Bionics

Bionics is the design and construction of mechanical, electrical, or optical devices to replace or supplement natural tissues and organs. Advances in materials science, engineering concepts and microelectronics in the latter half of the 20th century have led to the development of a number of highly successful bionic replacements and supplements, such as artificial joints, cardiac valves and pacemakers.[6-8] These prostheses are well-tolerated by the body, rarely experience structural failure and restore a large measure of functional normality.

However, there are a number of problems associated with bionic devices.[9] Even the best metal alloys and plastics available cannot match the properties of natural tissues. Body fluids corrode these materials, releasing fine particulates which elicit an inflammatory response, causing the implants to lose their attachment to the surrounding tissue. The current 10 to 20 year life expectancy of bionic devices, while fine for older persons, means multiple surgical replacement procedures for younger individuals who receive them. Finally, we are as yet unable to engineer devices to replace such vital organs as the heart, kidney and lung that can match the size, form and function of the natural organs.

Solid organ transplantation

Autotransplantation is an ancient practice described over a thousand years ago by the Indian physician, Sushruta, in a procedure for rebuilding damaged noses and earlobes.[10] Successful allogeneic (between genetically different individuals of the same species) solid organ transplants began with kidneys in 1954. Today, as our understanding of the immune system has grown, almost every solid organ can be successfully transplanted either singly or in combinations. The advantage of a solid organ transplant is its morphological, structural, and functional identity to the original. The five-year survival rate is high, approaching 90% for kidney transplants.[11] Some patients have lived nearly 30 years with kidney transplants, apparently having developed peripheral tolerance for the donor organ.

The major drawbacks to solid organ transplants are an acute shortage of donors[12] and the need to use immunosuppressants to prevent rejection of the transplant. Current immunosuppressants such as cyclosporine A, azothioprine and steroids have a variety of undesirable side effects, including musculoskeletal disorders, vascular disease, hypertension, diabetes, and leaving the body open to infection.[11] Solving the problem of immune rejection could potentially solve the donor shortage problem as well, by making it possible to use animals as donors. Such xenotransplants normally do not survive because they are subject to complement-mediated hyperrejection within minutes or hours following transplantation.[13] Thus, contemporary research on solid organ transplantation is directed at finding ways to evade the immune system without immunosuppression.

REGENERATIVE BIOLOGY

Regenerative biology is the science of creating new tissues, either in vitro or in vivo, to replace or regrow damaged tissues. Regenerative biology encompasses three major research approaches: transplantion of cells which will form new tissue in the transplant site, implantation of bioartificial tissues constructed in vitro and stimulation of regeneration from residual tissues in vivo.[14,15] All of these approaches usually involve the partial

† This paper, based on a lecture to the Minnesota Academy of Science, is dedicated to the memory of my father on the 39th anniversary of his death, April 18, 1958.

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recapitulation of embryonic development. Thus, regenerative biology draws heavily on the principles and concepts of developmental biology.

Cell transplantation

In humans, cell transplants have been used clinically to successfully repair articular cartilage of the knee and as therapy for Parkinson's disease. Cells have been transplanted experimentally in animals as potential treatments for Alzheimer's disease and for the replacement of damaged cardiac muscle.

Brittberg et al.[16] have described the autoplastic transplantation of chondrocytes to fill in limited full-thickness defects of knee joint articular cartilage. In their technique, a small biopsy of cartilage is taken from the injured surface by arthroscopy, cultured and chondrocyte clones expanded in vitro. Dissociated chondrocytes from these clones are then implanted under a flap of periosteum sutured over the lesion site. Within the defect, new cartilage is formed that stains with metachromatic dyes and with fluorescent antibodies to type II collagen. Although the new cartilage is presumably derived from the transplanted cells, its origin from chondrocytes at the edges of the defect or from osteogenic cells of the periosteal flap has not been ruled out.

Parkinson's disease results from the death of dopaminergic neurons in the substantia nigra that project to the putamen and caudate (basal ganglia) that, in turn, project to the motor cortex and control motor function. Neurons of the putamen and caudate require dopamine from the substantia nigra to fire properly, so death of substantia nigra neurons results in loss of motor control and the muscle rigidity and tremor characteristic of the disease. Suspensions of dopaminergic ventral mesencephalic cells from 8-9 week fetuses have been injected into the putamen of patients to treat severe Parkinson's disease.[17] Dopamine production by the transplanted cells was assessed by PET (positronic emission tomography) scan following the injection of 6L-18F-fluorodopa. Five months after transplantation, dopamine levels in the putamen had increased and symptoms of the disease were dramatically alleviated.

Cholinergic tissue from a variety of sources (fetal basal forebrain, nodose ganglion, intracortical adrenal chromaffin cells and differentiated neuroblastoma cells) survived when injected into the terminal areas of the forebrain cholinergic projection system of rat models of Alzheimer's disease. The transplanted neurons exhibited axonal outgrowth, formed extensive synapses with host neurons, and significantly reversed deficits in cognitive behavior.[18-20]

To test the feasibility of using cell transplants to replace damaged cardiac muscle, suspensions of fetal cardiomyocytes from mice bearing a lac-Z reporter transgene driven by the cardiac heavy myosin promoter were injected into the uninjured ventricular myocardium of genetically identical hosts.[21] The transplanted cells proliferated and differentiated into mature cardiac muscle, as indicated by the expression of the lac-Z reporter. Electron microscopic analysis showed that the differentiated cells formed intercalated discs, suggesting their probable electrical coupling with host cardiac myofibers. Similar results have been obtained after transplanting fetal cardiomyocytes into the ventricular myocardium of dogs.[22]

None of the transplanted cells described above elicited an immune response because none were "seen" by the immune system. The donor cartilage cells were autogeneic (from the same individual), the donor cardiac cells were syngeneic (from a genetically identical individual) and the brain is an immunoprivileged site. To fully exploit the potential of cell transplants, we need to be able to evade the immune system and to solve the problem of a reliable source of donor cells, just as with solid organ transplantation. In addition, there are difficult to resolve ethical issues associated with the use of fetal tissues as sources of transplantable cells. Recently, mouse embryonic stem (ES) cells have been used as a renewable source of cardiomyocytes for injection into the ventricular myocardium of *mdx* dystrophic mice.[23,24] The transplanted cells were stably integrated into the host cardiac muscle as judged by anti-dystrophin immunostaining. The use of a human ES cell line as a source of cardiomyocytes would avoid the ethical controversies associated with transplantation of fetal cardiomyocytes, but such a cell line is not yet available.

Bioartificial tissues

Another way of restoring tissues is to construct them in vitro, a process often referred to as "tissue engineering." The construction of a bioartificial tissue requires both a cell source and a supporting matrix that will either maintain the functions of differentiated cells seeded into the matrix or promote the differentiation of embryonic precursor cells. The ideal supporting matrix is biodegradable so that it is replaced over weeks and months with the natural matrix made by the cells. Collagens are the predominant proteins of support tissues in the body. Collagen I is plentiful, and easy to extract and mold into a variety of forms. It is thus the most widely used matrix biomaterial for both bioartificial tissues and stimulation of regeneration in vivo either by itself or in combination with various glycosaminoglycans such as chondroitin 6-sulfate. Other widely used matrix materials are alginate, polydioxanone, poly(epsilon-caprolactone), poly(glycolic acid), and poly(lactic acid). All, except alginate (in crosslinked form), are biodegradable. Some matrix materials, such as poly(lactic acid) fibers, can be molded into the shape of the tissue or organ to be constructed. The cells of a bioartificial tissue can be autogeneic or allogeneic in origin. The advantage of allogeneic cells (assuming that the immune rejection problem can be solved) is that they can be cultured and banked in advance for use in cases where there is not enough time to expand a patient's own cells to construct the tissue. Autogeneic cells have the advantage of not eliciting an immune response.

The immune system can be evaded by a bioartificial tissue implant if it is constructed as a closed system; i.e., if the cells are isolated by a nonbiodegradable matrix with a pore size that allows nutrient and gas exchange but excludes T-cells and antibodies. For example, a closed bioartificial pancreas has been clinically tested in a 38-year-old male patient who had been diabetic for 30 years.[25] This construct consisted of high-guluronic acid alginate microcapsules containing islet cells harvested by collagenase digestion from eight cadavers. The patient received a total dose of 15,000 encapsulated islets/kg body wt implanted into the intraperitoneal cavity in two doses of 10,000 and 5,000 islets six months apart. Three months after the second implantation, diabetic symptoms were markedly abated and the patient was able to discontinue insulin injections. Proinsulin levels, however, were borderline, suggesting that the dose of islet cells/kg body wt should be increased. How long the structural integrity of the microcapsules can be maintained and how long the islet cells can survive within the capsules are two important questions that have not yet been answered.

Regeneration in vivo from residual tissues

Mechanisms of natural regeneration

Regeneration from residual tissues in vivo would avoid all the disadvantages of bionic implants, organ and cell transplants and bioartificial tissue and organ implants, because the new tissue would be autogeneic and regrow with the same histological and morphological architecture as the original.

Regeneration in vertebrates is accomplished by three mechanisms. The first is the activation, proliferation and differentiation of progenitor or stem cells set aside during embryonic development for growth and regeneration later in life. Well-known examples of such reserve cells are the osteogenic cells of the periosteum and endosteum which regenerate bone, the satellite cells which regenerate skeletal muscle, the pericytes of capillaries which regenerate smooth muscle, and the epithelial stem cells, hematopoetic stem cells and bone marrow mesenchymal stem cells which maintain tissue integrity in the face of continual cell turnover. Recently, stem cell populations have also been found in the brain, liver, and pancreas.[26-28] The function of the stem cells in vivo is unknown, but if these tissues turn over slowly, the stem cells might be involved in replacing them, although there is no conclusive evidence for such turnover. Liver stem cells are known to be activated when hepatocyte destruction is so extensive (e.g, by carbon tetrachloride poisoning) that the liver cannot regenerate by compensatory hyperplasia of hepatocytes. The connective tissue compartments of all tissues are postulated to contain mesenchymal stem cells, since a subpopulation of connective tissue cells from a wide variety of tissues is capable of multipotent differentiation into fibroblasts, osteocytes, chondrocytes, fat cells and skeletal muscle when treated with dexamethasone.[29] These cells, too, might replace tissue that turns over slowly. However, their relationship to progenitor cells such as osteogenic and satellite cells is unclear and there is no evidence they act as progenitor or stem cells for regeneration in vivo after injury. In addition, the range of phenotypes they form in vitro is not appropriate for regeneration of some tissues such as cardiac muscle.

The second mechanism of vertebrate regeneration is to create progenitor cells from differentiated cells by a process of tissue matrix degradation and dedifferentiation of the liberated cells. Liver regeneration is accomplished by dissolution of the scanty liver tissue matrix and the minimal dedifferentiation of hepatocytes, which enables them to reenter the cell cycle while maintaining all their differentiated functions.[30] In mammals, the liver is the only tissue known to regenerate by partial dedifferentiation. The urodeles (salamanders and newts), however, are unique among vertebrates in that they use dedifferentiation to provide progenitor cells for the regeneration of a number of tissues that other vertebrates are unable to regenerate, including the neural retina, lens, cardiac muscle and complex structures such as the upper and lower jaws, limbs and tails. Following injury or amputation, the extracellular matrix (ECM) of these tissues is degraded at the site of damage, liberating the cells of the tissue. The liberated cells lose their phenotypic characteristics and revert to embryonic-like blastema cells. These cells reenter the cell cycle and proliferate to form a growing blastema, which subsequently redifferentiates into the missing structures. Interestingly, although the bone and muscle of urodele limbs contain osteogenic and satellite progenitor cells that are the source of regenerated bone and muscle following a nonamputational injury, there is no direct evidence that these cells participate in the regeneration of appendages following amputation.

The third mechanism is used to regenerate the spinal cord in urodeles, an ability which is unique among vertebrates.[31] In urodele spinal cord regeneration, the ependymal cells lining the lumen of the cord undergo an epithelial to mesenchymal transformation. The mesenchymal cells proliferate to bridge the lesion. In addition, they provide an environment favorable for the regrowth of severed axons and the establishment of functional synapses on either side of the lesion.

Stimulation of regeneration where it does not occur naturally

There are two reasons, not mutually exclusive, why most human tissues fail to regenerate. First, these tissues may have cells capable of engaging in regeneration but their environment may not contain the signaling molecules that stimulate regeneration or there may be inhibitory signals that divert the cells into a repair pathway. This includes situations in which regeneration may occur under one set of physical circumstances but not another, such as a bone fracture vs. regeneration of bone across a large gap. Alternatively, nonregenerating tissues may have the appropriate molecular environment for regeneration but may lack cells capable of responding to this environment. In either case, the tissues cannot recapitulate their embryogenesis. Thus, achieving regeneration of damaged tissues in vivo requires recreation of the embryonic environment in terms of cells, signaling molecules, or both.

A current strategy to stimulate regeneration in cases where it does not occur naturally is to bridge lesions with natural or artificial matrices that promote the migration, proliferation and differentiation of local regeneration-competent cells, or promote the regrowth of severed axons. There has been some success in applying this strategy to the regeneration of bone, dermis and spinal cord.

Although bone will regenerate to restore continuity after a fracture, it is unable to regenerate across a gap, such as that created by surgical removal of a bone tumor, probably because the sources of osteogenic cells, the periosteum, endosteum and bone marrow do not have the proper substratum to migrate and fill the gap. Calcium phosphate-based hydroxyapatite matrices promote bone regeneration.[32-34] The freshly made matrices are malleable pastes and can be pressed into a defect where they harden into a scaffold capable of temporarily substituting for bone. These scaffolds are osteoconducting as opposed to osteoinducing; i.e., the matrix supports the migration of osteoblasts from the host periosteum and endosteum rather than inducing osteogenic cells to become osteoblasts. The migrating cells differentiate and deposit natural bone matrix that gradually replaces the hydroxyapatite scaffold.

Hydroxyapatites have a high affinity for bone morphogenetic proteins (BMPs, members of the transforming growth factor-beta [TGFβ] family) and osteoinductive matrices have been prepared by adsorbing BMP fractions onto porous hydroxyapatites. This BMP-hydroxyapatite composite induces rapid bone differentiation in skull defects of adult baboons.[34] Only porous hydroxyapatite in block form, as opposed to particulate hydroxyapatite, is effective, showing the importance of matrix geometry in bone induction. Other growth factors known to be involved in skeletogenesis (other members of the TGFβ family, insulin-like growth factor [IGF]-I, platelet-derived growth factor [PDGF], or fibroblast growth factor [FGF]), or

combinations of these factors may be equally important in inducing large-scale bone regeneration and need to be tested.

Biodegradable artificial matrices can induce dermal regeneration in excisional skin wounds, but regeneration is imperfect. These matrices typically consist of collagen or gelatin sponges to which other ECM components, such as chondroitin 6-sulfate or elastin, are added.[35-38] The matrix is overlaid with either a meshed split-thickness epidermal graft or a suspension of dissociated keratinocytes. Fibroblasts from the surrounding dermis migrate into the artificial matrix and replace it with a matrix that resembles, but is not identical to, normal dermal matrix. These results clearly show that, given the appropriate environment, the adult dermis has the capacity for regeneration. However, it is not known whether the responding cells are the same dermal fibroblasts that normally form scar tissue or a subpopulation of regeneration-competent reserve cells that are normally suppressed in favor of repair. There is some evidence, based on differences in thymidine incorporation and pattern of cell association in vitro, that hypodermal fibroblasts form the granulation tissue of repair whereas dermal fibroblasts might be the cells capable of regeneration.[39] The presence of an artificial dermal matrix in a wound might suppress proliferation of hypodermal fibroblasts and promote proliferation of dermal fibroblasts, leading to regeneration. The nature of the factors that suppress either dermal fibroblast proliferation after wounding or hypodermal fibroblast proliferation in the presence of an artificial dermal matrix is unknown but could be investigated in vitro with an artificial matrix system.

Fetal skin regenerates perfectly. Several studies suggest that differences between fetal and adult skin in the proportions of ECM molecules and the relative concentrations of TGFβ isoforms are important in determining whether a wound scars or regenerates.[40-42] Changing the relative concentrations of TGFβ isoforms of mouse fetal skin toward those of the adult, and vice versa, increases and decreases scarring, respectively.[43,44] Thus fetal skin is an important model for the design of an artificial dermal matrix to promote the perfect regeneration of adult skin.

Regeneration of cut peripheral nerve axons in mammals is promoted by factors resident in the Schwann sheath.[45,46] Mammalian spinal cord axons, however, do not regenerate, even though they have the intrinsic capacity to do so. Their failure to regenerate is due to inhibitory factors such as toxic proteins in degenerating myelin, oligodendrites, and axonal surfaces, calcium influx and associated toxic reactions, glutamate excitotoxicity, cholesterol depletion (arachidonic acid pathway) and astroglial scar formation.[31,47] Toxic axonal damage can be prevented by cell membrane-protective agents such as methyl-prednisolone and lazaroids, which increase the chances for functional recovery in humans if administered within the first eight hours after injury to the cord.[48,49]

Functional axonal regeneration across spinal cord lesions in neonatal rats occurs after transplanting fetal cord to bridge the lesion, as demonstrated by anterograde and retrograde labeling of axons with fluorescent dyes and by recovery of righting reflexes and walking ability.[50] Partial functional recovery was achieved in adult rats by bridging cord lesions with grafts of intercostal nerves stabilized by a fibrin matrix impregnated with FGF-1.[51] Rats could support their weight on the previously paralyzed hindlimbs and displayed movement in all leg joints, but did not recover coordinated locomotion. Inclusion of FGF-1 in the matrix was essential; no functional regeneration took place in rats implanted with intercostal nerve and fibrin alone.

Our ultimate goal is to stimulate the regeneration of spinal cord and other tissues using artificial matrices designed to neutralize inhibitory factors and to exactly reproduce the molecular environment which guided the initial development of that tissue.

Contemporary and Future Research Directions

Cell sources

Providing reliable sources of cells for cell transplants and bioartificial tissue construction is a crucial issue that requires establishing culture banks of proliferating stem, progenitor, or differentiated cells which can be drawn upon as required. These cells can be in their natural condition or be genetically engineered to produce desired molecules. Many types of differentiated cells, such as hepatocytes and islet cells, are difficult to expand in vitro. Stem and progenitor cells are difficult to identify and purify. Furthermore, not all the factors essential for their proliferation and differentiation are understood. Thus, research on the factors that control the entry of stem, progenitor and differentiated cells into and progression through the cell cycle and the factors that control the differentiation of specific cell phenotypes will be of prime importance for the design of media that allow cell expansion and differentiation. For example, a defined medium containing hepatocyte growth factor (HGF), epidermal growth factor (EGF), and TGFβ-1 has recently been found to support the proliferation of mature rat and human hepatocytes.[52] The stem cells harbored by the adult rat brain were identified as stem cells because they proliferate in vitro in response to EGF to form neurospheres.[26,53] Single cells of these neurospheres plated in medium without EGF will differentiate into neurons, astrocytes, or oligodendrocytes.[53,54] The ability to culture these stem cells and differentiate them into the three major cell types of the central nervous system makes them a potentially unlimited source for neural cell transplantation.

Materials science

Advances in materials science are essential for advances in bionics, construction of bioartificial tissues and for stimulation of regeneration in vivo.

Metal alloys and plastics that do not corrode, that have the strength and plasticity of natural tissues, and which have surfaces that do not trigger thrombosis will result in great improvements in the function and durability of bionic implants. The brightest future may be in the development of sensory devices such as artificial cochleas and retinas, electronic blood pressure regulators and devices that both sense and regulate concentrations of physiologically important molecules such as glucose.

New biomaterials will be required for the construction of better bioartificial tissue matrices and matrices that stimulate regeneration in vivo. Such matrices must meet several criteria to be useful. They must be "smart"; i.e., contain or be able to release appropriate biological signaling information to promote and maintain cell adhesion, differentiation and tissue organization. In essence, we want to develop polymers that mimic the functions of the embryonic or adult ECM. Thus, research on polymer chemistry must be coupled with research on the molecular biology of ECM signaling systems that regulate cell morphology and function. For example, adhesive oligopeptide or carbohydrate recognition sequences might be linked to artificial polymers. Selectivity of cell adhesion to substrates could be achieved by linking specific recognition sequences to nonadhesive polymers.

Such adhesive selectivity would be desirable, for example, in artificial blood vessels, where the materials used should support the adhesion and migration of endothelial cells but not the adhesion of platelets, which could trigger thrombosis.[55] Growth factors might also be bound to polymer matrices and released as the polymers degrade.[56] If the matrix materials form a closed system, they must allow the ready exchange of oxygen, nutrients, waste products and molecules that regulate the metabolic and synthetic activities of the cells, while immunoprotecting cells.

Directional alignment is another "smart" matrix property that would be useful in bioartificial tissue construction or regeneration in vivo. Controlled alignment of the elements of the biomaterial would allow it to orient cells, by contact guidance, in particular directions essential for the function of the tissue. For example, circumferential orientation of smooth muscle cells (which would be important for construction or regeneration of tubular tissues such as blood vessels) entrapped in a forming collagen matrix has been achieved in vitro by using a strong magnetic field to orient the collagen fibrils as they form.[57] Copolymers of collagen I and chondroitin 6-sulfate in which the pore channels were aligned longitudinally produced superior regeneration of peripheral nerves within guide tubes compared to other directions of alignment.[45]

One of the most important properties of matrix materials is controlled biodegradability. The most widely used matrix material, collagen I, is susceptible to rapid degradation in vivo and, therefore, is thermally or chemically crosslinked to increase its stability before use. Glutaraldehyde is the most commonly used chemical crosslinking agent because it provides the highest stability. However, biomaterials crosslinked with glutaraldehyde also induce cytotoxicity. Recent experiments suggest that collagen I crosslinked with carbodiimide or acyl azide is more biocompatible than collagen I crosslinked by glutaraldehyde.[58] Only a few other biodegradable polymers, such as poly(glycolic acid), poly(lactic acid), polydioxanone and their copolymers, are available today to make artificial matrices. These polymers have the disadvantages of being relatively stiff and lacking chemically active side chains for easy attachment of biologically active molecules, crosslinkers, or drugs. New matrix biomaterials, based on tyrosine-derived polycarbonates and polyarylates, have been devised in which the backbone, side chain composition and length can be varied to change thermal behavior, mechanical properties, hydrophobicity and cell-polymer interactions.[59] These materials are hydrophobic and degrade slowly, over months to years. Currently, they appear to be most useful in situations where a long-term degradation profile is desired, such as in bone regeneration.

Experimental biomaterials are also being investigated that will undergo phase transformation at body temperature.[60] For example, outside the body such a material might be a liquid that occupies only a small volume, but when injected into the body it would transform at the higher temperature into a semisolid or solid. If the transformation involved expansion in volume, only small apertures would be required to implant large constructs.

Evasion of the immune system

Wider use of cell transplants and bioartificial tissue implants, particularly open constructs, will require the ability to avoid immune rejection. Several innovative and promising methods have been explored for evasion of the immune system without compromising its function. Donor transgenic animals have been created which express immune regulatory molecules that inhibit the activation or synthesis of key proteins involved in rejection. One of these is human decay acceleration factor (hDAF) which inhibits the hyperacute rejection caused by the endothelial cell damage and vascular ischemia triggered by activation of complement.[61] Immunosuppressed monkeys, which would normally hyperreject donor pig hearts within a matter of minutes, survived for a median of 40 days after receiving pig hearts transgenic for hDAF.[62]

Another strategy has been to mimic immunoprivileged sites such as the anterior chamber of the eye. Transplants are not rejected at these sites because cytotoxic T-cells entering them are killed. T-cell apoptosis is mediated by a protein, FasL, produced by cells at the immunoprivileged site which binds to the Fas receptor expressed on T-cell surfaces.[63] Syngeneic myoblasts transfected with the FasL gene were cotransplanted with allogeneic pancreatic islet cells under the kidney capsule of mice rendered diabetic with streptozotocin.[64] These fused to form myotubes expressing FasL which killed infiltrating T-cells, thus preventing rejection of the islets. The production of animals carrying transgenes that regulate or inhibit the immune system is also a potential way to solve the problem of renewable sources of donor cells, tissues and organs.

Finally, perhaps the most intriguing strategies to pursue are the production of antibodies directed at T-cell receptors which recognize foreign cell surface major histocompatibility antigens (MHCs) and the genetic engineering of "stealth" cells that do not express surface MHC antigens. In either case, T-cells would be blind to foreign cells transplanted alone or as part of a bioartificial construct.

Stem and progenitor cells; stimulatory and inhibitory factors for regeneration in vivo

A number of questions about reserve cells needs to be answered. How ubiquitous are such cells in mammalian tissues? Do they exist in every tissue? What are their identifying characteristics that will enable us to recognize them? What is their normal function in tissues that do not regenerate in response to injury? Do the tissues in which they are located turn over slowly and do these cells function to replace the cells that turn over?

If stem or progenitor cells exist in every body tissue, but are inhibited from participating in regeneration in most tissues because of the presence of inhibitory molecules or the absence of the appropriate stimulating molecules, the question of stimulating regeneration becomes one of determining how to identify and neutralize inhibitors, or of identifying and applying stimulators of regeneration to nonregenerating tissue. One way to identify these stimulatory and inhibitory molecules might be to construct cDNA libraries from regenerating tissue mRNA and subtract them with mRNA from uninjured tissue or tissue that responds to injury by repair. The subtracted libraries would then be transfected into cells, such as Chinese hamster ovary (CHO) cells, that would express and secrete the proteins encoded by the cDNAs. The conditioned medium from the culture of aliquots of these cells in multiple wells could then be tested on bioassy systems for their regeneration-promoting ability. Positive samples would be sequenced and their human counterparts identified for use as regenerative agents.

Mechanical load (tension, compression) is an important factor which triggers the synthesis and/or release of molecular signals important to regeneration, differentiation and hypertrophy, particularly in musculoskeletal tissues. In some

vertebrates, like the chicken or rat, muscle can regenerate from a remaining short muscle stump. In the rat, for example, regeneration from the stump of the gastrocnemius muscle is correlated with the regeneration of an Achilles tendon which connects to the regenerating muscle. The tension exerted on the muscle stump by the Achilles tendon is thought to be an important factor in regeneration of muscle.[65]

Mechanical stress causes hypertrophy of cardiac and skeletal muscle and increases collagen synthesis by cardiac fibroblasts. In human skeletal muscle tissue the hypertrophy is associated with increased release of FGF-2.[66] In cultured fetal rat cardiac fibroblasts the increase in collagen synthesis is correlated with enhanced responses to TGFβ and IGF-1.[67] The transition from tendon to fibrocartilage in bovine deep flexor tendon is associated with increased expression and accumulation of two proteoglycans, aggrecan and biglycan. The levels of TGFβ and of mRNAs for these proteoglycans are increased by compression of fetal tendon, suggesting that fetal tendon cells respond to compression by increasing TGFβ synthesis which, in turn, stimulates production of proteoglycans characteristic of fibrocartilage.[68]

Dedifferentiation

What if tissues do not regenerate because they lack reserve cells? In this case, we would like to decipher the urodele technical manual to understand how to create progenitor cells out of differentiated cells via histolysis and dedifferentiation. Histolysis and dedifferentiation have been most extensively studied in the regenerating limbs of salamanders and newts. After amputation of a limb, the tissue matrix at the amputation site, which is predominantly composed of collagens, elastins and sulfated proteoglycans, is degraded and replaced with a more embryonic-like matrix that is much richer in fibronectin, hyaluronic acid, and tenascin. The degradation is accomplished by lysosomal acid hydrolases, particularly acid phosphatase, and members of the matrix metalloproteinase (MMP) family of which 11 have been identified in mammals and chickens. MMP-9 (gelatinase b; hydrolyzes gelatin, proteoglycan, and collagen types IV, V, VII, and X) is upregulated within a few hours of amputation in regenerating axolotl limbs[69] and as early as two days in the adult newt limb[70]. In both axolotl and newt, its expression declines after formation of a conical blastema. MMP3/10a and MMP3/10b (stromelysins 1/2; degrade fibronectin, laminin, proteoglycan, and collagen types II, IV, V, IX, and X) are also strongly upregulated as early as two days in regenerating newt limbs. MMP3/10a is weakly transcribed up to 8 days postamputation then disappears by 15 days, which is when blastema cells are just beginning to accumulate; whereas expression of MMP3/10b continues through the palette stage[70]. The different expression patterns of these three MMP genes suggests that they may have different roles in degradation of limb ECM. Acid phosphatase is most strongly expressed during the periods of dedifferentiation and blastema formation.[71] The cell types producing these proteases, however, have not yet been identified.

The cells liberated from degrading matrix lose their internal specializations, become mesenchyme-like, and reenter the cell cycle. Little is known about the mechanisms of this dedifferentiation. The act of liberation from mature ECM itself, which likely involves breaking of ECM ligand-cell surface receptor contacts, may play a role in starting the process. Tenascin and two other anti-adhesive proteins, SPARC (osteonectin) and thrombospondin, promote reorganization of the actin cytoskeleton in bovine arterial endothelial cells by modulating adhesive contacts.[72] Whether these proteins might function to reorganize the cytoskeleton of amputated urodele limb cells (particularly myofibers) is not known.

The cells of healing mammalian wounds also employ proteases to remodel ECM. Like the regeneration blastema, the early wound matrix is dominated by hyaluronate and fibronectin but, unlike the blastema, it is replaced not by normal matrix but by a matrix enriched in crosslinked collagen I. With the exception of the minimal dedifferentiation exhibited by hepatocytes in regenerating liver, mammalian cells are not known to dedifferentiate in vivo after injury, although some cell types, such as chondrocytes, will dedifferentiate under certain in vitro conditions. Mammalian tissues, thus, would appear to react differently to injury than urodele limb tissues. The nature of these differences is largely unknown but could involve the ionic and molecular composition of the injury environment, intrinsic differences in the responses of the cells to that environment, or both.

Recently, Tanaka, et al.[73] have demonstrated what appears to be an intrinsic difference between cultured mammalian and newt myotubes in their response to high concentrations of serum. Nuclei of cultured mammalian myotubes do not synthesize DNA, regardless of the concentration of serum to which they are exposed. Nuclei of cultured newt myotubes do not synthesize DNA in low (5%) concentrations of serum but do so at a 20% serum concentration. DNA synthesis is correlated with phosphorylation of the retinoblastoma (Rb) protein by cyclin-dependent kinase 4/6, which causes it to dissociate from E2F transcription factors, allowing these factors to activate genes that drive the nuclei into S phase of the cell cycle.[74] Thus, newt myotubes appear to have heightened sensitivity to serum factors that lead to phosphorylation of Rb. The nature of this sensitivity is not known, but is not due to retention of sensitivity to FGF-2, EGF, or other standard growth factors such as PDGF and IGF, since DNA synthesis was not elicited by these growth factors. The response is dependent, however, on the degree of contact of myotubes with other cells. High-density cultures of myoblasts surrounding the myotubes prevents them from synthesizing DNA whereas low density cultures do not.[73] This suggests that contact inhibition normally inhibits newt myotubes from seeing or responding to stimulatory factors in serum and that amputation and matrix remodeling relieves this inhibition. Much research remains to be done to understand the process of dedifferentiation and reentry into cell cycle by urodele cells participating in regeneration, how these events might be linked and the differences between these cells and those of healing mammalian wounds.

Finally, we need to understand how limb blastema cells are maintained in a dedifferentiated state and kept in the cell cycle. Serum factors that cause newt myotube nuclei to synthesize DNA are not sufficient to drive them through the G2/M transition.[73] Other factors, not yet uneqivocally identified, provided by wound epidermis and nerves are essential for complete cell cycling.[14] The embryonic-like ECM is thought to be instrumental in maintaining blastema cells in an undifferentiated state,[75] probably by the nature of the contacts between cells and matrix. The wound epidermis is also known to be essential for maintaining cells in a dedifferentiated state,[76] although whether this is by its effect on cell cycling or is an independent effect is unknown. The homeobox gene *Msx*-1 is expressed in blastema

cells[77] and may be instrumental in repressing genes responsible for differentiated function. Forced expression of *Msx*-1 in mouse myoblasts downregulates *MyoD,* which maintains terminal muscle differentiation and prevents them from differentiating.[78]

Concluding Statement

Currently, we are very limited in what we can do to replace tissues with cell transplants or bioartificial constructs, and in our ability to stimulate the regeneration of tissues in vivo. But the basic research approaches are now in place to make real advances. Not too far into the 21st century, we may realistically expect to be able to regenerate a number of vital tissues in a clinical setting.

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