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Review

Complement-triggered pathways orchestrate regenerative responses throughout phylogenesis

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ABSTRACT

Adult tissue plasticity, cell reprogramming, and organ regeneration are major challenges in the field of modern regenerative medicine. Devising strategies to increase the regenerative capacity of tissues holds great promise for dealing with donor organ shortages and low transplantation outcomes and also provides essential impetus to tissue bioengineering approaches for organ repair and replacement. The inherent ability of cells to reprogram their fate by switching into an embryonic-like, pluripotent progenitor state is an evolutionary vestige that in mammals has been retained mostly in fetal tissues and persists only in a few organs of the adult body. Tissue regeneration reflects the capacity of terminally differentiated cells to re-enter the cell cycle and proliferate in response to acute injury or environmental stress signals. In lower vertebrates, this regenerative capacity extends to several organs and remarkably culminates in precise tissue patterning, through cellular transdifferentiation and complex morphogenetic processes that can faithfully reconstruct entire body parts. Many lessons have been learned from robust regeneration models in amphibians such as the newt and axolotl. However, the dynamic interactions between the regenerating tissue, the surrounding stroma, and the host immune response, as it adapts to the actively proliferating tissue, remain ill-defined. The regenerating zone, through a sequence of distinct molecular events, adopts phenotypic plasticity and undergoes rigorous tissue remodeling that, in turn, evokes a significant inflammatory response. Complement is a primordial sentinel of the innate immune response that engages in multiple inflammatory cascades as it becomes activated during tissue injury and remodeling. In this respect, complement proteins have been implicated in tissue and organ regeneration in both urodeles and mammals. Distinct complement-triggered pathways have been shown to modulate critical responses that promote tissue reprogramming, pattern formation, and regeneration across phylogenesis. This article will discuss the mechanistic insights underlying the crosstalk of complement with cytokine and growth factor signaling pathways that drive tissue regeneration and will provide a unified conceptual framework for considering complement modulation as a novel target for regenerative therapeutics.

1. Introduction – overview

1.1. Current trends and challenges in regenerative medicine

Regenerative biology defines a rapidly expanding field of research that comes to terms with the very essence of organismic development; the inborn ability of cells and tissues to reprogram their fate, switch into an embryonic-like, pluripotent state, and repopulate damaged or malfunctioning organs through lineage-specific redifferentiation [1].

Regenerative responses culminate through finely orchestrated cellular processes and fate-deciding molecular circuits that are activated in response to perturbations attempting to "dismantle" tissue homeostasis. Unraveling the overarching signals, genetic and epigenetic factors, and cellular mechanisms that are recruited by tissues in order to activate their complex regenerative programs essentially amounts to understanding the evolutionary trail of cellular pluripotency, lineage-specific commitment, and cell differentiation [2,3]. The ontogenetic pathway that a cell follows resembles to a great extent the developmental blueprint of the entire organism. In this respect, the early development of all mammals proceeds through a sequence of fate-deciding stages along an irreversible pathway of restricted plasticity and increasing specialization.

The long-prevailing dogma that cell differentiation is a unidirectional process irreversibly leading to the formation of distinct

Abbreviations: ECM, extracellular matrix; DAMPs, danger-associated molecular patterns; HSCs, hematopoietic stem cells; HSPCs, hematopoietic stem-like progenitor cells; C3aR, C3a receptor; C5aR, C5a receptor; MSCs, mesenchymal stem cells; EMT, epithelial-to-mesenchymal transition; IPE, iris pigmented epithelial; MAC, membrane attack complex; RPE, retinal pigmented epithelium; CCl_4, carbon tetrachloride; PHx, partial hepatectomy; ASP, acylation-stimulating protein.

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and rigid tissue blueprints has been drastically challenged over the years through the discovery of pluripotent adult stem cells [4]. Since the seminal observations of *John Gurdon* and his nuclear transfer experiments in *Xenopus laevis* that eloquently demonstrated that the nucleus of a terminally differentiated cell can be reprogrammed epigenetically and give rise to a fertile mature organism [5], stem cell biology has expanded in new directions, paving the way for the advent of adult cell reprogramming technologies, tissue bioengineering, and regenerative medicine [6].

Regenerative medicine applies tissue bioengineering and cell replacement technologies to address the mediocre regenerative capacity of adult tissues and counteract the shortage of donor organs and generally dismal outcomes of organ transplantation. It offers great promise for ex vivo organ development through manipulation of the adult stem cell potential to drive tissue repair and regeneration processes [6,7]. Crucial to our understanding of the molecular basis of tissue plasticity and adult organ regeneration are insights provided by regeneration models developed in various phylogenetically distant species, such as flatworms (planarians), urodele amphibians, and rodents [8]. Through persistent natural selection processes, nature has taught us that the regenerative potential of adult tissues regresses significantly as the evolutionary scale draws closer to higher vertebrates. Regeneration remains in mammals as a vestigial activity that is evident only in a few fetal tissues during embryogenesis [9].

A hallmark of tissue regeneration in lower vertebrates is the elaborate tissue reconstruction program that involves coordinated morphogenetic rearrangements, dedifferentiation of adult tissue-specific cells, and precise whole-body patterning that can tailor amputated limbs to regenerate the entire structure of a fully functional organ [10]. In sharp contrast to urodele species, the regenerative responses of mammals lack the pluripotency of tissue patterning and proceed through a rather linear pathway of tissue-compensatory hyperplasia, leading to mass restoration of damaged tissues/organs (e.g., liver regeneration) [11]. Several studies have highlighted the important contribution of stem-like progenitor cells to the tissue regenerative process [12]. It is now well appreciated that stem-like progenitor cells that reside within the damaged tissues become activated by a wide array of factors released in the microenvironment of the regenerating tissue and assume the ability to differentiate along various cell lineages [13]. In several cases, these pluripotent cells are thought to act in conjunction with other parenchymal or non-parenchymal stromal cells to coordinate the regenerative response. Exceptions to this rule apply to several regeneration models in which the role of stem cells is still debatable, such as in models of liver regeneration in which parenchymal cell division accounts for tissue restoration.

Tissue regeneration culminates in the activation of multiple growth factor-triggered pathways that drive cell cycle re-entry and proliferation of previously quiescent and terminally differentiated cells of the adult body [1]. However, as with many other biological processes, the pathway of tissue regeneration is far from being unidirectional. Regenerative responses involve the concerted action of multiple biological systems and proceed through the activation of resident cells and of stem-like progenitors that either reside locally in the tissue or become mobilized and home to the tissue after being generated in the bone marrow or other peripheral organs [14]. It is now a prevalent concept in the field that tissue regeneration can manifest itself only under the dynamic interaction of stem-like, tissue-committed, stromal and immune cell-derived factors [15]. The integrity of this diverse network of interactions is thus a prerequisite for the fine execution of a tissue regenerative program.

From a therapeutics standpoint, modern bioengineering endorses the concept of a systems-wide impact on regenerative responses and aims to optimize strategies for ex vivo tissue regeneration and whole-organ replacement [7]. Biomaterial-based scaffolds are being developed as biocompatible supports for tissue reconstruction, taking into account the various spatiotemporal constraints and factors affecting cell self-renewal and coordinated proliferation. The essential elements that have to be mounted together in an ex vivo system for organ regeneration include the reprogrammed adult stem cell pool, a source of nourishing growth factors, and a biocompatible 3D-scaffold upon which cell–cell contact and interactions will be promoted [12]. These bioengineering rules ensure that proliferating cells will develop a 3-D network resembling the actual architectural tissue pattern of the intact organ [7]. Decellularized matrices based on collagen and other extracellular matrix (ECM) constituents essentially provide the regenerating cells with all the stimulants (growth factors) and regulators that will coordinate the regenerative process [16]. However, a significant limitation in further developing ex vivo systems for tissue engineering lies in the absence of a fully functional immune system that will monitor the growing tissue, as well as a lack of immune effector cells that normally infiltrate the regenerating tissue to support the cell–cell interactions that drive tissue repair and regeneration [15]. Recent studies have given new momentum to the field by highlighting the essential role of immunomodulators, such as inflammatory cytokines and innate immune pathways (i.e., complement) in the early stages of regeneration [11,17]. Accumulating evidence suggests that there is a dynamic interplay between the immune response and various tissue repair and regeneration programs. However, the fine interactions that twine together the inflammatory and regenerative cascades are yet to be fully elucidated.

1.2. Tissue regeneration, immunity, and inflammation: tales of mutual attraction?

The remarkable ability of invertebrates such as flatworms and lower vertebrates to regenerate entire body parts has been tightly linked to the presence of an immature and permissive immune system that lacks basic aspects of acquired immunity and thereby allows the promiscuous growth of regenerating tissues in the absence of tissue immunosurveillance, lymphocyte activation, and histocompatibility constraints [8,18]. In these organisms, the lack of a fully developed acquired immune response is counterbalanced by the presence of a versatile and multifaceted innate immune system that exhibits broad-range immune recognition properties [19]. Indeed, the gradual decline of regenerative potential along the evolutionary ladder leading to mammals and the modest or absent regenerative capacity of adult tissues in humans underscore *a relationship of reciprocity between the state of immune competence and an organism's regenerative potential* [20]. Several studies have highlighted a mutual interdependence of the immune system and regenerative processes, indicating that immunity may act as a double-edged sword in modulating the outcome of regenerative responses [15]. Moreover, the influence of inflammation on the regenerative program of various species often appears to be context-driven [15].

In this respect, studies in *Xenopus* employing limb amputation models have suggested that the local inflammatory response elicited upon formation of a post-amputation wound exerts an inhibitory effect on the regenerative capacity of the remaining tissue [20]. Through the developmental transition of young larvae to adult organisms and in later stages of *Xenopus* maturation and metamorphosis, the regenerative potential of developing tissues regresses significantly following the gradual maturation of the immune system [21].

On the other hand, recent evidence from lens regeneration studies in salamanders (newts) argues for a favorable role of inflammation and immunity in the regulation of regenerative responses. Induction of a local inflammatory response in the

lens after surgical incision leads to activation of innate immune responses that, in turn, promote lens regeneration from the dorsal iris [22]. Interestingly, dendritic cell transfer from injured eye structures of the newt into normal recipients evokes a de novo regenerative response in the recipient's lens and promotes newt lens fiber formation [22], thus implying an essential role for local inflammation in the induction of lens regeneration.

Tissue regeneration entails a significant component of cell turnover and remodeling in response to injury or other environmental insults (i.e., physical or toxic chemical agents). The early stages of the regenerative process closely resemble the closure of a wound and scar formation through extensive tissue remodeling [23]. Rigorous tissue remodeling involves protease-mediated degradation of extracellular matrices and the dissolution of basement membranes to facilitate coordinated cell migration. Matrix rearrangements, through intense cell turnover, induce the local release of danger signals (DAMPs) that can activate pattern recognition receptors of the innate immune response [24]. Local inflammation enters a vicious cycle that is perpetuated by the ongoing tissue remodeling activity during regeneration and triggers the release of other immunomodulatory factors (cytokines, chemokines) that activate innate immune pathways and mediate the local activation (polarization) of inflammatory myelomonocytic cells [15]. Furthermore, it is now evident that the local inflammatory milieu within an injured organ produces signaling effectors that exert multiple effects on both local and distal cell populations, which then act in concert to promote the regenerative process. The essential contribution of hematopoietic stem/progenitor cells, mesenchymal stem cells, circulating endothelial progenitors, and stromal cells to tissue regeneration and repair is now widely acknowledged [12,14]. Indeed, it is the chemotactic arsenal, fueled by local inflammation, that coordinates the directional migration of stem/progenitor cells to the regenerating zone from distal sites such as the bone marrow or spleen. The dynamic interplay between the mobilized stem cell compartment, the inflammatory infiltrate, and the tissue stroma and parenchyma is critical for the timely activation of the regenerative program.

In the early stages of tissue repair, leukocytes are stimulated to extravasate from the bloodstream, transmigrate through endothelial barriers, and infiltrate the regenerating tissue [25]. Neutrophils are among the earliest innate immune effectors to become attracted to the site of injury by a wide array of chemotactic signals. Neutrophils then amplify the local inflammatory milieu by producing a wide array of immunostimulatory molecules that modulate critical aspects of the repair and regeneration processes. These factors include proinflammatory cytokines (IL-1β, IL-6, TNF-α), adhesion molecules (P-selectins, VCAM, ICAM), and chemokines important for stem cell recruitment (SDF-1α, IL-8, MCP-1, etc.) [25]. Signals produced during the repair and regeneration processes can also direct the migration of blood-derived monocytes into the tissue [15]. Under the influence of local immunostimulatory signals, these monocytes differentiate into mature tissue macrophages and become polarized toward an inflammatory M1 or immunosuppressive M2 phenotype [26]. These cells are considered crucial effectors of the healing and regeneration processes through their ability to secrete a plethora of growth factors (including TGF-β, bFGF, PDGF), cytokines, and proangiogenic stimuli (i.e., VEGF). Their capacity to switch between "polarization" states has also been postulated to affect the regeneration process [15,26].

Effective revascularization of regenerating organs is vital for the restoration of organ function [14]. Neoangiogenesis is an integral aspect of tissue regeneration that supports tissue outgrowth and involves the coordinated activation of several cell types, including resident endothelial cells and bone marrow-derived endothelial progenitors [27]. Inflammatory pathways provide essential signals for the activation of the proangiogenic switch during tissue regeneration. VEGF and other proangiogenic factors have been shown to be secreted by activated tissue macrophages and also from bone marrow-derived myelomonocytic cells that are recruited in response to chemotactic gradients propagated by the local tissue inflammatory milieu [15,28].

Several lines of evidence suggest that inflammatory circuits and, in particular, innate immune systems such as complement, play a vital, fine-tuning role in the processes of tissue repair and regeneration. Complement proteins have been shown to modulate critical elements of the regenerative response in both lower vertebrates and mammals [17,29]. Complement-triggered pathways appear to forge dynamic interactions with the stem cell compartment, the vascular stroma, and the parenchyma of regenerating tissues, and through crosstalk with cytokine and growth factor-mediated signaling pathways drive tissue regeneration across phylogenesis.

1.3. Complement: a multifaceted gatekeeper of tissue homeostasis

Complement is a phylogenetically conserved effector of the innate immune response that has co-evolved with blood-borne microbial defense systems that mediate direct cytolytic and opsono-phagocytic activities against invading pathogens [30]. Complement-related activities and protein homologs can be traced back to the early branches of invertebrate chordate evolution: the urochordates (tunicates) and also more primitive deuterostomes such as echinoderms (sea urchins) [19,31].

Complement components participate in various immunoregulatory circuits via a complex and dynamic network of protein–protein interactions that culminates in the formation of multi-protein complexes with distinct enzymatic activities [30]. The complement cascade comprises a wide array of soluble glycoproteins, membrane-bound receptors, and fluid-phase or membrane-anchored regulatory proteins. Upon complement activation via any of the three canonical pathways (classical, lectin, alternative), a well-orchestrated sequence of protein–protein interactions is initiated that results in the proteolytic cleavage of precursor molecules, the release of bioactive peptides (such as the anaphylatoxins C3a and C5a from cleavage of the main complement effectors C3 and C5, respectively), and the downstream activation of receptor-mediated signaling pathways in complement-targeted cells [30]. Recently, a novel "extrinsic" pathway of complement activation was described that is initiated by direct cleavage of complement C3 and C5 components by serine proteases of the coagulation cascade, in a convertase-independent fashion [32].

Traditionally, the complement system has been associated with antimicrobial defense and pathogen-containment mechanisms that culminate in direct pathogen elimination or antibody-mediated opsonophagocytosis of targeted cells. However, several studies have provided insights into novel functions of complement that extend far beyond its immunomodulatory profile [33]. Complement components have also been implicated in the clearance of tissue turnover byproducts (e.g., cell debris, apoptotic cells) and play a vital role in initiating and regulating multiple proinflammatory pathways in the context of health and disease [34,35]. Complement components are mainly produced in the liver as acute-phase reactants. However, the discovery of several extrahepatic sites of complement expression in both parenchymal and non-parenchymal tissues has indicated that complement might mediate distinct fine-tuning roles in the context of tissue homeostasis and immunosurveillance [30]. The ability of tissues to synthesize their own arsenal of complement proteins and to regulate complement biosynthesis in a hormone- or growth factor-dependent manner has prompted speculation that local complement might function in a context-driven fashion, independent of its systemic activities. Indeed, a series of fascinating findings over the years has

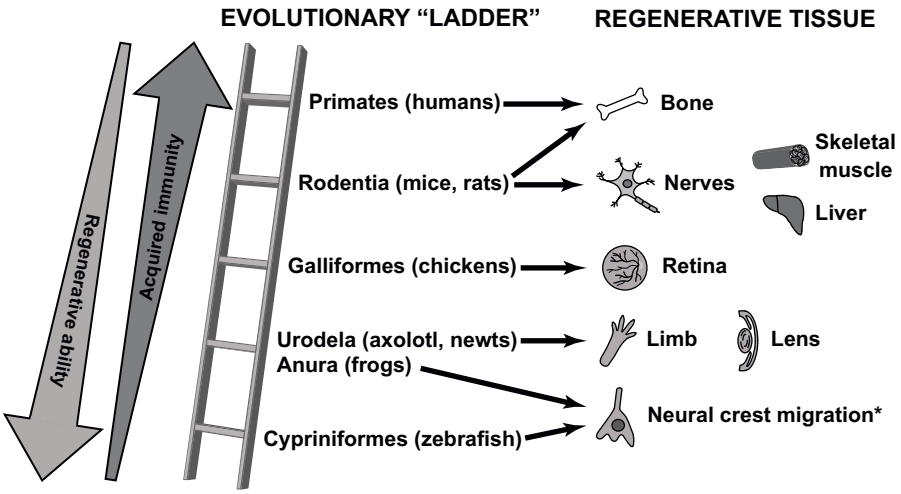

Fig. 1. Complement-associated regenerative processes in evolutionarily distinct organisms. Taxonomic order ranks are shown as an evolutionary "ladder" from lower-order species such as zebrafish up to humans. As one ascends the ladder, there is a gradual maturation of immune competence through the development of a fully operational and diversified branch of acquired immunity. Conversely, the regenerative potential of organisms is low in higher-order animals and increases as one goes down the ladder. The specific regenerative processes for each taxonomic order in which complement is known to play a role, as discussed in the text, are shown. *, Zebrafish and frog neural crest migration is listed due to the multipotent embryonic-like nature of the neural crest cells, though complement has been shown to regulate this process during development, not tissue regeneration.

illustrated various unconventional roles of complement-triggered pathways in developmental and homeostatic processes across phylogenesis. It is of particular note that complement components have been shown to affect regenerative responses in a broad spectrum of animal models employing species that are phylogenetically divergent. Fig. 1 summarizes the regenerative processes in which complement has been shown to play a role and integrates them in an evolutionary backdrop that highlights the interplay between an organism's regenerative capacity and state of immune competence/maturation.

1.4. Complement modulation of stem/progenitor cell biology

The stem cell compartment (hematopoietic progenitors and mesenchymal stem cells) actively contributes to the tissue regenerative program by responding to multiple growth factor-dependent, inflammatory, and chemotactic signals that guide the egress of cells from distant sites and their homing to regenerating tissues [4,36]. Hematopoietic stem cells (HSCs) are a population of progenitors found primarily in the bone marrow that can replenish all mature blood lineages; these cells serve as a typical source for marrow engraftment in patients with hematologic malignancies. In addition, HSCs have been implicated, either through paracrine signaling or perhaps transdifferentiation, in the regeneration of various tissues [14]. HSCs are a rare population in the bone marrow (and the placenta), and strategies for isolating and expanding these progenitors are of great therapeutic potential [36,37]. Mesenchymal stem cells are also generated in the bone marrow and define a population of pluripotent progenitors that can give rise to mesenchymal tissues such as osteoblasts, chondrocytes, and adipocytes [38]. Accumulating evidence suggests that harvesting and manipulation of these progenitor cells for therapeutic purposes might also augment repair and regenerative responses after tissue injury [14].

Recent studies have unraveled fascinating roles for complement in modulating stem cell biology. Strikingly, these complement-modulated interactions appear to be very relevant to the successful implementation of a tissue regeneration program. Complement anaphylatoxin receptors (C3aR, C5aR, C5L2) are expressed in hematopoietic stem-like progenitor cells (HSPCs) and myeloid precursors, and C3aR engagement on CD34+ HSPCs has been shown to modulate SDF-1-dependent homing responses and to promote hematopoietic cell regeneration after myeloablative treatment [39,40]. Exposure of HSCs to complement activation fragments appears to modulate their interaction with the bone marrow stroma by increasing CXCR4-dependent retention in the bone marrow niche and affecting cell adhesion and MMP-dependent matrix degradation [41]. In this context, accelerated stem cell mobilization in response to G-CSF treatment has been demonstrated in $C3^{-/-}$ and $C3aR^{-/-}$ mice, suggesting that pharmacologic blockade of the complement C3a-C3aR axis might be an intriguing strategy to increase the release of HSPCs into the periphery for stem cell transplantation [42]. Such a complement-targeted approach might also be worth exploring in the context of regenerative responses for promoting the homing of hematopoietic progenitors into damaged or regenerating organs. C3aR interception might augment the release of progenitors into the periphery, while C3aR activation might promote the trafficking and homing of progenitors into the tissue by sensitizing cells to a local SDF-1 gradient.

Mesenchymal stem cells (MSCs) are considered a versatile, highly migratory and pluripotent cell population whose manipulation offers great promise for tissue repair and regeneration after injury (e.g., organ ischemia, infarction) [38]. These cells are readily mobilized in response to perturbations that affect tissue homeostasis and rapidly respond to chemoattractants and other immunomodulatory signals. Recent studies have provided evidence for a pivotal role of complement activation in regulating MSC responses. The anaphylatoxins C3a and C5a appear to contribute to the recruitment of MSCs to distant tissues by acting as chemotactic factors for human bone marrow-derived MSCs, which express both C3aR and C5aR [43]. Furthermore, MSCs display complement-activating properties and complement has been shown to modulate critical interactions of MSCs with other immune effector cells in a proinflammatory context [44]. Moreover, complement anaphylatoxin-triggered pathways have been linked to vital developmental processes that involve coordinated progenitor or mesenchymal stem cell migration. Frog (*Xenopus*) and zebrafish (*Danio rerio*) neural crest cells, a multipotent embryonic cell population that rapidly undergoes an epithelial-to-mesenchymal transition (EMT) and exhibits a highly migratory phenotype during embryogenesis, have been shown to respond to C3a by directing collective cell migration in a C3aR-dependent manner [45]. Furthermore, neural progenitor cells express complement anaphylatoxin

receptors, and C3 deficiency or blockade of C3aR signaling has been shown to attenuate basal and ischemia-induced neurogenesis in the adult mouse brain [46]. The effects of C3a on basal neurogenesis are mediated through its synergy with SDF-1α in promoting neural progenitor cell migration and differentiation [47].

Taken together, these fascinating findings suggest that complement activation and distinct bioactive fragments modulate previously unchartered stem cell functions in early vertebrate development that are highly relevant to the molecular circuitry underlying tissue regeneration. This unprecedented crosstalk between complement proteins and the stem cell compartment opens new conceptual avenues for the rational design of regenerative therapies that might exploit complement modulation as a novel target for stem cell mobilization and homing into regenerating tissues.

2. Tissue regeneration in lower vertebrates

Comparative phylogenetic studies have been instrumental in furthering our understanding of the molecular basis of organ regeneration [9,48]. Regeneration appears to be a primordial attribute of metazoans that has gradually been lost or constrained during phylogeny, as organisms evolved to more complex and energy-demanding structures [8]. Along this trail of evolution, urodele amphibians hold a unique place in view of their capacity to regenerate entire body parts through precise tissue patterning and elaborate morphogenetic processes [10]. Systematic investigation of regenerative responses in diverse models and pathological contexts has revealed that tissue regeneration in lower vertebrates is largely dependent on lifelong tissue plasticity, rather than on mobilizing a pool of self-renewing stem cells on demand [1]. Regenerative responses rely on the ability of adult differentiated cells to escape quiescence and dedifferentiate into pluripotent precursor cells that eventually reprogram their genome and differentiate into the various lineages comprising the regenerating tissue.

A molecular hallmark defining the early stages of regeneration in lower vertebrates is the ability of tissue-specific cells to transdifferentiate into divergent cell phenotypes in the absence of remaining organ structure, topological information, and developmentally regulated paracrine or endocrine signals [48]. This attribute is remarkably illustrated in newt lens and retina regeneration models, in which existing adult cells of epithelial or neural/glial origin shed their differentiated phenotype, reprogram their transcriptional signature, actively proliferate, and transdifferentiate into specialized cells (lens fibers or retina cells) of the regenerated structure [49].

Teleost fish also possess noticeable regenerative capacities in various organs [9]. The zebrafish serves as an ideal model system for understanding vertebrate embryonic development, partly because of its fascinating regenerative capacity in various tissues [50]. Like salamanders, adult zebrafish effectively regenerate multiple structures, including retinas, brain tissues, spinal cord, and major appendages (fins) [14]. Zebrafish display the most robust cardiac regenerative response known to date [14,50]. In heart ventricular resection models, zebrafish have been shown to respond to injury by forming a fibrin clot that is not replaced by scar tissue during cardiac repair. This wound region is gradually replaced by a zone of actively proliferating cardiomyocytes, and persistent cardiac regenerative activity over several weeks restores the full function of the injured heart [14]. Inflammation has been postulated to contribute to the early phases of zebrafish heart regeneration through injury-related inflammatory triggering [15]. In contrast to the high regenerative index of zebrafish cardiac cells, adult mammalian cardiomyocytes display very poor proliferative responses to injury (e.g., myocardial infarction), and the limited extent of heart repair relies invariably on the recruitment of cardiac stem-like progenitors and mesenchymal stem cells to the infarcted tissue.

It should be stressed that partial regenerative responses have been recorded in mammalian species; however, this attribute is present only during fetal development and regresses in adulthood. In most cases, adult tissue regeneration resembles hyperplastic responses that rely on remnant cell proliferation rather than dedifferentiation, cell reprogramming, and transdifferentiation processes.

2.1. The urodele regeneration paradigm

Urodele amphibians have long been considered the “masters” of regeneration because of their promiscuous ability to regenerate tissues by orchestrating complex morphogenetic and developmental programs that culminate in the faithful reconstruction of entire body parts [48]. Indeed, an adult newt can effectively regenerate a broad spectrum of organs and appendages, including its limbs, tail, jaws, lens, retinas, and large portions of its intestine, brain, and heart tissue [10].

Limb regeneration in axolotls offers a striking paradigm of how regenerative responses can evoke adult cell plasticity and drive dynamic tissue patterning and morphogenesis. Moreover, limb regenerative responses provide an excellent conceptual framework for better understanding the origins and establishment of positional identity in adult vertebrate cells [1]. After amputation of the limb, a specialized wound epithelium is formed that progressively becomes covered by epithelial cells (“wound epidermis”). Unlike lens regeneration, in which epithelial cells take charge by initiating transdifferentiation processes, in limb regeneration the main effector population that rebuilds the entire structure is a zone of mesenchymal cells located underneath the wound epithelium that re-enter the cell cycle and proliferate. After limb amputation, cartilage, connective tissue, and muscle cells begin to lose their phenotypes and dedifferentiate to form a mass of mesenchymal stem/progenitor cells that make up the regenerating and pluripotent blastema [48,51]. Activation of rigorous matrix remodeling, blastema cell proliferation, morphogenetic rearrangements, and subsequent differentiation of blastemal cells into essentially all cell types (bone, muscle, cartilage) underscore the main stages of the regenerative response in the urodele limb [48]. Remodeling of the ECM is of profound importance to the successful outcome of the dedifferentiation process leading to blastema formation. Many cell adhesion proteins and proteolytic enzymes such as integrins, collagens, and collagenases are expressed specifically during this stage and mediate critical interactions with serum and matrix-derived factors [18].

Newt lens and retina regeneration models represent the most extensively studied blueprints of adult cell reprogramming and transdifferentiation in lower vertebrates [49]. Dorsal iris pigmented epithelial (IPE) cells can regenerate the entire newt lens following lentectomy, through a process of transdifferentiation into lens cells (lentoid bodies) [49]. During the early stages post-lentectomy, IPE cells dedifferentiate, losing their pigmentation, and gradually adopt a highly proliferative index. In subsequent stages, IPE cells change morphology, begin to synthesize lens-specific proteins, and differentiate into lens fibers and lens epithelial cells. In contrast, other amphibian species such as *Xenopus* have lost the capacity for lens regeneration in adulthood but still retain this ability during earlier developmental stages [52]. *Xenopus* lens regeneration involves the transdifferentiation of corneal epithelial cells into lens vesicles and requires a close interaction of the cornea with neural retinal factors and other ECM matrix-associated growth factors [49]. It should be noted that extensive ECM remodeling activity regulates the dedifferentiation of adult cells into pluripotent progenitors in lower vertebrates. For example, the dedifferentiation of IPE cells

during newt lens regeneration has been partly attributed to the upregulated expression of remodeling enzymes (e.g., collagenases, hyaluronidases) in the newt dorsal and ventral iris [53,54]. In stark contrast to urodeles and *Xenopus*, lens regeneration in mammals appears to be incomplete and deviates from the maintenance of a physiological structure [49].

2.2. Role of complement in urodele limb and lens regeneration

2.2.1. Complement in limb regeneration

Limb regeneration in urodeles recapitulates the key stages of embryonic development by integrating cell reprogramming, dedifferentiation, morphogenesis, and complex pattern formation. These critical processes require extensive ECM remodeling to facilitate cell locomotion and directional migration toward the apex of the wound epithelium [1,10]. Such pathways can trigger the local release of danger signals that instruct innate immune effectors to release immunostimulatory signals and coordinate early inflammatory responses [55]. Moreover, it has been postulated that monocytic cells infiltrate the blastemal zone underneath the wound epithelium, acting as sentinels that protect the exposed wound and remnant tissue from potential pathogens or environmental insults. It is intriguing to speculate that innate immunity might provide essential signals for the chemotactic recruitment of such cells to the blastema–wound epithelium interface and also uphold tissue immunosurveillance during the regenerative process.

Complement, as a pivotal mediator of inflammation and a primordial sentinel of innate immunity in lower vertebrates, has been investigated as a putative regulator of the regenerative response in urodeles in studies probing the spatiotemporal expression of complement components in axolotl and newt limb regeneration. C3 was found to be expressed in limb structures during blastema formation, in the wound epithelium and in the undifferentiated blastema [56]. In later stages of limb regeneration, C3 was localized preferentially to the presumptive muscle and cartilage regions of the regenerating limb. Comparative analysis of C3 expression in normal (not injured) limbs indicated that significant C3 expression is selectively localized to the ectoderm or mesenchymal cell compartment only in the regenerating limb. To corroborate these findings, *Tsonis and colleagues* went on to demonstrate that C3 is specifically expressed by blastema cells of myogenic origin [56]. These cells have the ability to differentiate into myotubes and support muscle and cartilage development when engrafted into the amputated limb [57]. These studies provided unprecedented evidence for a novel role of complement C3 in limb regeneration that deviates from its traditional proinflammatory profile. The tissue distribution profiles of C3 intriguingly imply that C3 biosynthesis or activity is linked to the pivotal process of cell reprogramming and dedifferentiation and appear to polarize pluripotent blastema cells toward the myotube lineage. In line with these findings, complement C5 has also been shown to be differentially expressed during limb regeneration [58]. A spatiotemporal profile of C5 expression has revealed its exclusive presence in the wound epithelium of the amputated limb, suggesting a role for C5 in the wound healing process.

Further supporting an essential role for complement in amphibian regeneration, *Prod1* has been identified as a novel gene that is differentially expressed in newt blastema cells under the influence of retinoic acid and proximodistal positioning during limb regeneration [59]. Prod1 is a GPI-anchored protein and the newt ortholog of mammalian CD59, a membrane regulator of the lytic complement pathway that leads to membrane attack complex (MAC) formation. These findings provide further credence to complement's involvement in amphibian regeneration and implicate Prod1/CD59 in cell–cell interactions that define the positional identity of regenerating cells.

2.2.2. Complement in lens and retina regeneration

The eye is an immunoprivileged organ that has developed distinct immunosurveillance mechanisms [60]. Urodeles possess striking plasticity and regenerative capacity in the lens and retina structures of the eye [49]. In conjunction with their role in limb regeneration, the complement proteins C3 and C5 appear to also modulate lens regeneration in urodeles. In a newt lentectomy model, C3 and C5 expression has been found to share striking complementarity in spatial distribution during lens regeneration [58]. C3 is localized in the stroma and pigmented epithelium of the dorsal iris, while C5 mRNA and protein selectively accumulate in the regenerating lens vesicle and cornea. These findings suggest that complement proteins might regulate key regenerative processes leading to lens vesicle formation. In view of the fascinating spatiotemporal profile of complement expression in the newt lens, one might also speculate on the yet elusive functions of complement components in tissue remodeling and pattern formation in the eye.

During avian embryogenesis, retina regeneration can be triggered through reprogramming of the retinal pigmented epithelium (RPE) or by activation of retinal stem/progenitor cells present in the anterior margin of the eye [61]. A recent study employing a chick retina regeneration model has implicated complement C3 and C5 components as well as C3aR and C5aR signaling in embryonic eye development and retina regeneration [86]. A distinct spatiotemporal profile of complement expression was recorded in intact embryonic eyes and in eye structures undergoing retina regeneration. Strikingly, both C3a and C5a were found to promote chick retina regeneration in a growth factor-independent manner, via transdifferentiation of RPE cells or stem/progenitor cell recruitment.

3. Complement in mammalian regenerative processes

3.1. Insights from liver regeneration models

The mammalian liver is among only a few organs of the adult body that have retained the inherent ability to regenerate in response to acute toxic injury, viral infection, or surgical resection (hepatectomy) [11]. Liver regeneration culminates in the coordinated activation of growth factor-regulated and cytokine-driven pathways that instruct quiescent hepatocytes and other non-parenchymal liver cells (Kupffer cells, endothelial cells) to re-enter the cell cycle and proliferate [11]. The potential contribution of hepatic stem cells/progenitors to the regenerative process has attracted considerable attention in recent years but still remains controversial [13]. In general, stem cells are thought to play a role in the restoration of liver mass only when hepatocytes are unable to proliferate, possibly in response to the presence of toxins or other insults. Hepatocyte regeneration leads to the restoration of liver mass and metabolic integrity through a pathway of compensatory hyperplasia that does not involve cell reprogramming and dedifferentiation, at least to the extent observed in lower vertebrates. Nonetheless, the regenerative capacity of the liver has been the subject of intense investigation because emerging insights from its molecular regulation might offer new perspectives and unprecedented opportunities for therapeutic interventions in liver transplantation, such as prolonging donor graft survival and protecting livers from ischemic damage.

The liver is well equipped with resident inflammatory effectors, partly because it is continually exposed to potential blood-borne

pathogens and toxins. However, the role of innate inflammatory pathways in triggering liver regenerative responses had for many years remained elusive, only finally beginning to be revealed during the last decade.

Prompted by the fascinating finding that complement proteins participate in multiple facets of urodele limb regeneration, a thorough investigation of complement's involvement in liver regeneration has been pursued in various animal models. The complement cascade was first implicated in liver regeneration by acute hepatotoxicity studies demonstrating the defective regenerative response of C5-deficient livers after exposure to carbon tetrachloride (CCl_4) [62]. Genetic deficiency of C5 was associated with defective re-entry of hepatocytes into the cell cycle, attenuated mitotic activity, and extensive liver parenchymal necrosis. Reconstitution of the liver with C5 or C5a restored the regenerative index and significantly protected mice from acute liver damage. Blockade studies using a specific C5aR antagonist revealed the pathway by which C5 mediated its protective effect, implicating C5aR signaling as an essential participant in this process [62]. Later studies demonstrated a critical role for C3 and its active fragments in liver regeneration after CCl_4 injury, showing that both $C3^{-/-}$ and $C3aR^{-/-}$ mice had a defective response and that C3a reconstitution could effectively restore the liver regenerative phenotype [63]. A two-wave complement activation profile (i.e., C3b/iC3b/C3c levels) was prominent in sera from CCl_4-treated mice. These studies provided evidence for a dual role of C3 activation during liver regeneration after CCl_4 injury: C3a generation contributes to the early priming of hepatocytes, whereas a second wave of C3 activation at later times after CCl_4 treatment contributes to the clearance of injured tissue.

The essential role of complement as a modulator of the signaling network promoting liver regeneration was unequivocally established in a murine partial hepatectomy (PHx) model. Studies in this model showed that genetic deficiency of both C3 and C5 resulted in attenuated hepatocyte proliferation after PHx, and combined C3a and C5a reconstitution restored regeneration and mitigated parenchymal necrosis in $C3/C5^{-/-}$ mice [64]. A biphasic complement activation profile was evident in PHx-treated mice, in line with the liver toxicity studies mentioned above. Inhibition of C5aR signaling abrogated IL-6/TNF-α induction, and the lack of C3aR and C5aR stimulation attenuated NF-κB/STAT-3 activation after PHx [64]. These studies furnished a mechanism for complement's involvement in liver regeneration, suggesting that local complement activation within the portal circulation leads to C3a/C5a generation and that subsequent engagement of their respective receptors on hepatic Kupffer cells modulates the cytokine response leading to hepatic regeneration. An IL-6-C5aR activation loop was also suggested as a means of further priming C5aR-positive Kupffer cells to release IL-6, and C5aR signaling was linked to activation of key hepatic transcriptional networks that drive cell cycle re-entry (STAT3 and NF-κB). A role for the alternate C5a receptor, C5L2, and its putative ligand acylation-stimulating protein (ASP; C3adesArg) in the regulation of liver regeneration and injury-induced steatosis was recently demonstrated [65]. ASP administration reduced liver injury and steatosis in $C3^{-/-}$ mice and augmented hepatocyte proliferative responses following PHx. Furthermore, $C5L2^{-/-}$ mice displayed increased mortality, hepatic necrosis, and impaired liver regeneration after PHx, suggesting a key role for C5L2 in the regenerative process.

Complement deficiency resulted in an early wave of caspase-dependent parenchymal apoptosis during liver regeneration. The pronounced hepatoprotective effect of complement after PHx was mediated by an intact IL-6 signaling circuit, associated with early activation of STAT3/JAK1 and the induction of robust IL-6-dependent prosurvival signaling through the PI3K/AKT and mTOR pathways [66]. The proliferative effect of C3 on hepatocytes was also found to be pERK-dependent [66].

The mechanism of C3 cleavage in the regenerating liver remains debatable. Recent studies have shown that mice deficient in classical, alternative, or lectin pathway components retain normal regenerative responses after PHx, raising the possibility that C3 is activated during liver regeneration in a non-traditional manner, possibly via the extrinsic protease-mediated pathway [67].

New insights into the fascinating crosstalk of complement with the cytokine milieu driving liver regenerative responses were recently provided by a study revealing a complement-IL-4 regulatory feedback loop that controls liver regeneration [68]. IL-4 was shown to be a novel driver of liver regeneration, and IL-4 production after PHx was regulated by complement activation via NKT cell recruitment to the liver. Moreover, IL-4 appears to fine-tune complement activation in the regenerating liver through the maintenance of serum IgM and an increase in IgM deposition in the liver parenchyma after PHx.

3.2. Complement in cardiac and skeletal muscle regeneration

Heart regeneration has been a topic of rigorous investigation, given the overarching role of this organ in human physiology [69]. Heart failure, a leading cause of mortality worldwide, results from cardiac myocyte loss after extensive myocardial infarction. Myocardial infarction is associated with an inflammatory response, ultimately leading to healing and scar formation. The lack of clinical treatment, other than transplantation, that can replenish cardiac myocytes has prompted the search for noninvasive, stem cell-based, cardiac replacement therapies [50]. The plasticity of the fetal heart and the identification of progenitors with potency for cardiac differentiation have augmented efforts to develop regenerative therapeutic strategies [69]. Indeed, HSCs mobilized by G-CSF have been shown to repopulate the infarcted myocardium and to regenerate cardiomyocytes and coronary vessel-forming cells in mice [37].

Thus far, complement has mostly been assigned to the "dark side" of cardiac physiology because of its detrimental proinflammatory sequelae. Several studies have indicated that dampening complement activation ameliorates cardiac dysfunction and protects cardiac tissue from ischemia/reperfusion injury [70]. C5aR stimulation has been linked to sepsis-induced cardiomyocyte dysfunction [71], and the C5b-9 complex, which is known to form under conditions of ischemia, may contribute directly to myocardial cell injury [72]. However, the action of complement in muscle physiology might also be context-driven, and several indications point to a "hidden" role of complement-triggered pathways in muscle homeostasis and development.

In this respect, sublytic C5b-9 has been shown to induce c-fos-dependent myotube dedifferentiation in culture, suggesting that complement modulates the developmental program of skeletal muscle cells by inducing their switch into a pluripotent progenitor-like state [73]. Moreover, C3a and C5a have recently been implicated in the heart healing process, by modulating critical aspects of cardiac progenitor cell biology, and promoting the differentiation of cardiac resident stem cells along the myofibroblast lineage [85]. C3 expression in urodele blastema cells of the myotube lineage [56] further supports a phylogenetically conserved role for complement in regulating muscle cell plasticity and differentiation.

Prompted by emerging evidence that anaphylatoxin signaling modulates critical stem cell responses, including stem cell mobilization, and based on the observation that mobilized hematopoietic progenitors can repopulate the infarcted heart, it would be intriguing to speculate that complement modulation strategies (e.g., increased mobilization of HSCs employing specific C3aR antagonists) might open new avenues of opportunity for cardiac regenerative therapies. Complement could serve as a means of augmenting mobilization of bone marrow-derived progenitors

to the heart and also as a robust chemotactic stimulus for MSC recruitment to the infarcted myocardium.

An interesting paradigm shift concerning the role of complement in tissue regeneration was recently suggested in a study implicating C1q in aging-related impairment of muscle regeneration [74]. Wnt/β-catenin signaling has been implicated in multiple aspects of embryonic development, including tissue regeneration, tumorigenesis, and aging [75]. Complement C1q was shown to activate canonical Wnt signaling by binding to Frizzled receptors and inducing C1s-mediated cleavage of a Wnt coreceptor on myocytes. Activation of Wnt signaling by C1q mediated impaired skeletal muscle regeneration in aged animals, marked by increased muscle fibrotic responses (collagen deposition) after cryoinjury. This finding suggests that targeted C1q/C1s inhibition might promote muscle regeneration following injury or aging-related deterioration.

3.3. Complement and bone regeneration

Bone homeostasis is tightly connected to the immune system, and the crosstalk between bone and the immune system depends on the deployment of common immunomodulators that effectively regulate bone healing and resorption [76]. A paradigm of this complex interaction is illustrated by the recruitment of cytokines that promote osteoclast-mediated resorption and osteoblast-induced bone healing. In fact, numerous inflammatory mediators have been linked to bone metabolic and healing processes [76].

Several lines of evidence suggest that complement and the skeletal system forge mutual interdependencies that affect bone development. The initiating enzyme of the classical pathway, C1s, has been localized to hypertrophic chondrocytes within the primary ossification center of the femur, and its collagen-degrading activity has been linked to matrix degradation during endochondral bone formation [77]. Primary osteoblasts secrete C3 in response to 1α,2,5-dihydroxyvitamin D_3, and C3 also appears to synergistically promote M-CSF-dependent osteoclast differentiation from bone marrow progenitors [78]. These findings support a crucial regulatory function for C3 in osteoclast differentiation during bone formation [79]. A distinct spatiotemporal profile of C5aR expression was recently described in osteoblasts, osteoclasts, and chondroblasts involved in the healing of bone fractures [80]. Furthermore, C5aR expression was notably upregulated in human MSCs induced to differentiate along the osteogenic lineage, and C5aR stimulation induced osteoblast migration, suggesting that complement might modulate osteoblast and progenitor cell trafficking during bone repair.

A potential mechanism for local complement activation during bone remodeling was revealed by studies indicating that primary osteoblasts and osteoclasts can cleave C5 to generate active C5a [81]. Both C3a and C5a anaphylatoxins were shown to induce, in synergy with IL-1β, a pro-inflammatory response in osteoblasts, leading to expression of IL-6 and IL-8 [81]. IL-1β and anaphylatoxins also synergize to induce the production of the pro-osteoclastogenic cytokine RANKL and its competitor osteoprotegerin (OPG) in primary osteoblasts [81]. These findings indicate that complement may modulate the inflammatory state of osteoblastic cells and affect the osteoblast-osteoclast interaction by tilting the balance toward an osteoblastic or osteoclastogenic phenotype, depending on the local cytokine milieu and the bone pathology involved.

In conjunction with recent findings indicating that mesenchymal stem cells respond to C3a and C5a by adopting a highly migratory phenotype [43], these studies provide an integrative framework for elucidating the role of complement in bone regeneration after injury (e.g., fractures). Complement modulation (i.e., C3aR or C5aR activation) might be an attractive strategy for

Table 1
Overview of the various regenerative programs modulated by distinct complement components across phylogenesis.

Regenerative process	Species	Complement components involved	Target cell/tissue expression profile	Mechanisms/effectors	References
Limb regeneration	Urodele amphibians (axolotl, newt)	C3, C5, Prod1/CD59	Blastema cells, wound epithelium, prospective cartilage, muscle cells	Dedifferentiation, wound repair, muscle differentiation, positional identity?	[56,58,59]
Lens regeneration	Urodele amphibians (newt)	C3, C5	Iris pigmented epithelial cells, cornea, lens vesicle	Transdifferentiation, lens fiber formation?	[58]
Retina regeneration	Chicken (embryo)	C3, C5, C3a, C5a, C3aR, C5aR	Retinal pigmented epithelial cells, stem-like progenitor cells	Transdifferentiation of retinal pigmented epithelial cells, stem cell activation	Del-Rio Tsonis et al. (submitted)
Liver regeneration	Mouse	C3, C5, C3a, C3a*des*Arg, C5a, C3aR, C5aR, C5L2	Kupffer cells, hepatocytes, NKT cells	IL-4/IL-6/TNF-α release, NF-κB, STAT3 activation, PI3K/Akt, mTOR pathway activation	[62–66,68]
Skeletal muscle regeneration	Mouse	C1q, sublytic C5b-9	Mouse myoblasts, differentiated myotubes	Myotube dedifferentiation, Wnt/β-catenin activation, c-fos activation, ERK1 activation	[73,74]
Bone regeneration	Human, mouse, rat	C3, C1s, C3a, C5a, C3aR, C5aR	Osteoblasts, osteoclasts, chondrocytes, mesenchymal stem cells	Osteoclastogenesis, osteoblast migration-differentiation, IL-1β synergy, RANKL and OPG production	[77–81]
Peripheral nerve (axonal) regeneration	Rat	Sublytic C5b-9	Glial cells (Schwann cells)	Inhibition of apoptosis, cell proliferation, PI3K/Akt pathway activation	[83]
Central nervous system nerve (axonal) regeneration	Rat	Sublytic C5b-9	Oligodendrocytes	Prosurvival signaling, inhibition of apoptosis, PI3K/Akt pathway activation (regulation of Bad/Bcl-xL complex)	[84]

mobilizing bone-forming osteoblasts and mesenchymal progenitors to the injured bone to effectively promote bone regeneration.

4. Concluding remarks and future perspectives

Complement, a primordial sentinel of innate immunity, has long been perceived as a mere "executioner" that directly neutralizes pathogens or tags them to promote their phagocytosis. However, over the past two decades, complement biology has undergone a drastic reorientation by recapitulating old developmental paradigms from a systems-wide perspective. Emerging evidence from various animal models points to a more subtle role of this innate immune system in basic development, vertebrate embryogenesis, and tissue homeostasis. Complement proteins forge dynamic interactions with growth factor and cytokine-driven pathways in guiding stem cell compartments to mobilize or engraft more efficiently, and they also modulate critical cell responses that promote tissue regeneration after injury. Fascinating evidence has suggested that complement regulates amphibian regenerative responses and mammalian liver regeneration, as well as bone and muscle regenerative processes. Table 1 provides a comprehensive summary of the various regenerative programs modulated by complement and lists the underlying mechanisms and signaling effectors. Interestingly, complement extends its regulatory influence to critical aspects of central nervous system homeostasis and development [82] and also appears to promote nerve axon regeneration through interactions with glial cells (i.e., Schwann cells, oligodendrocytes) that mediate axon remyelination and motor function recovery after spinal cord injury [83,84]. As organismic evolution leads to more complex structures, the regenerative capacity deteriorates, and remains only as a vestige in fetal tissues. The interrelationship between immunity, inflammation, and tissue regeneration capacity has long remained obscure. It is of profound importance to note that the phylogenetically conserved complement system regulates tissue regeneration in diverse species. Studies across phylogenesis have revealed important mechanistic insights regarding complement's involvement in regenerative programs, and they provide a conceptual framework for exploiting complement modulation for the design of regenerative therapeutics and stem cell-driven tissue replacement strategies.

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