

Complement: An Inflammatory Pathway Fulfilling Multiple Roles at the Interface of Innate Immunity and Development

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Abstract: Complement has been long perceived as an innate immune system that plays a pivotal role in the maintenance of host defense against infectious agents and the propagation of pro-inflammatory responses in the context of human disease. Complement activation has been associated with the onset of acute inflammatory reactions leading to complications such as acute graft rejection, local tissue injury and multi-organ failure. However, recent studies have indicated that various complement activation products may exert a beneficial effect by contributing to critical developmental and regenerative processes. Appreciating this extraordinary 'versatility' of complement proteins provides a framework for revisiting the design of effective complement therapeutics. A balanced strategy will have to consider limiting the detrimental proinflammatory effects of complement while preserving those activities that promote tissue repair and regeneration, cell survival and early development.

INTRODUCTION

In the post-genomic era biomedical research is being revisited in the light of novel findings that introduce unexpected associations between otherwise divergent biological systems. Biological pathways are no more regarded as rigid entities that perform strictly defined functions in a 'linear' fashion. They rather appear to interact in a dynamic manner with other cellular and humoral partners to form intricate networks that converge and share molecular signals and ligands. Indeed, this is accurately illustrated by studies that apply combinatorial and cross-disciplinary approaches in assigning functions to specific molecules and focus on identifying functional associations and patterns in complex 'proteomes'. The complement cascade represents an example of such a system that has been recently associated with novel functions that go beyond the context of inflammation and traditional immunology.

Complement has maintained a high degree of phylogenetic conservation across the evolutionary ladder, having pre-dated the emergence of adaptive immunity [1]. It acts as a principal effector system in host defense against invading pathogens. It also contributes to the release of inflammatory mediators, promoting tissue injury at sites of inflammation, and has also been implicated in the pathogenesis of several autoimmune, ischemic and vascular diseases [2]. Moreover, it is now established that complement serves as a vital link between innate and acquired immunity by providing essential signals that augment the humoral response to antigens and affect the threshold of B-cell activation [3].

However, recent studies have provided evidence for alternative functions of complement that cannot be clearly placed in an 'inflammatory' context. Several complement components and activation products have been implicated as important mediators in processes ranging from human reproduction, bone and cartilage development to regeneration and stem cell engraftment [4]. Complement anaphylatoxins appear to modulate cell survival and regenerative pathways whereas until recently they were only associated with the detrimental consequences of inappropriate complement activation by triggering inflammatory responses that lead to tissue injury and organ damage [5].

These studies support the concept that complement may also contribute to such diverse functions and phenotypes through its functional 'crosstalk' with other cellular networks. Furthermore they provide a new platform for designing effective complement therapeutics, since it is now becoming evident that complement activation is far from being a harmful process that needs to be contained on an extensive and indiscriminating basis. A comprehensive approach consolidating these new findings is now more compelling than ever, in order to control the harmful inflammatory effects that lead to injury while preserving locally the activities that promote cell survival, remodeling and tissue regeneration.

This article discusses critical findings that have implicated complement proteins in non-inflammatory pathways and developmental processes. Human reproduction, tissue regeneration and hematopoietic development are used as striking paradigms of how complement activation may exert its beneficial effect in different physiological settings.

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COMPLEMENT COMPONENTS AS MEDIATORS OF REPRODUCTION AND EMBRYONIC DEVELOPMENT

For several years, a potential role for complement in human reproduction has remained elusive, despite the fact that a wide array of complement components and membrane regulators have been identified on almost all human reproductive epithelia as well as on the surfaces of sperm (e.g. MCP, CD59) and oocytes (e.g. MCP, CR1) [6, 7]. The prominent expression of complement factors has been associated with the protection of these immunologically privileged tissues from autologous complement activation and injury. Indeed, a recent study showing that *Crry*, a murine complement regulator, plays a critical role in maintaining feto-maternal tolerance during early pregnancy [8] has further highlighted the protective role of complement membrane regulators in preventing inappropriate complement activation at the placental interface. Interestingly, *Cry*-null mice lacking the C3 gene manage to rescue their fetuses from miscarriage and spontaneous abortion.

From a different standpoint however, several reports have pointed out that the biosynthesis of complement components and receptors in the reproductive tract is not constitutive but lies subject to fine hormonal regulation [6]. It appears that such tightly regulated expression, following stage-specific patterns during the menstrual cycle may influence normal reproductive processes. In support of this concept, it has been shown that estrogens can effectively stimulate C3 production by the glandular epithelium of the human and rat uterus. Furthermore, several components of the classical and alternative complement pathways are synthesized in the human and rat endometrium in a hormone-dependent manner, and distinct changes in the expression of various complement membrane regulators have been recorded during gamete maturation, follicular development, and ovulation [6].

Mammalian fertilization is a tightly regulated process that culminates through distinct spatio-temporal stages. These include, the sperm 'acrosomal reaction' that leads to the release of proteases into the sperm-oocyte interspace, the receptor-mediated attachment of the sperm on the oocyte membrane, and the sperm-oocyte fusion [9]. The potential involvement of complement in the early stages of gamete interaction and subsequent oocyte fertilization was strongly suggested in a study by Anderson *et al.* [10] It was shown that sperm acrosomal proteases released during the 'acrosomal reaction' are able to cleave C3, and that the C3-derived fragments, C3b and iC3b, bind to complement receptors present on the oocyte membrane in a way that secures the docking of the sperm and the subsequent fusion with the oocyte [10]. This study puts forward the intriguing concept that local complement activation in the human reproductive tract may promote sperm penetration and oocyte fertilization by releasing activated fragments that serve as a 'bridge' for the cross-linking of complement receptors on the gamete surface.

A role for C3 in human reproduction is further supported by a recent study that identifies iC3b as the potent embryotrophic factor ETF-3 that is secreted by human oviductal cells and enhances the development of the embryo at the stage of the blastocyst [11]. This study also demonstrated the presence of complement regulators such as *Crry* and CR3 in the mouse pre-implantation embryo, suggesting that upon local complement activation, oviduct-produced C3 is cleaved to C3b and further converted to iC3b in the presence of cofactors expressed on the surface of the embryo.

Taken together these studies highlight a previously under-appreciated role of complement in reproductive biology and clearly stress the concept that complement activation products may promote early embryonic development by facilitating fertilization and providing later on essential growth signals to the developing embryo. The fine balance between its harmful effects during pregnancy and its potentially beneficial role in reproduction may be dictated by distinct hormonal changes and a possible therapeutic potential of complement in terms of enhancing the reproductive capacity in humans remains to be determined.

COMPLEMENT IN TISSUE REGENERATION AND REMODELING

Limb Regeneration

Regeneration of entire body parts or complex structures is predominantly observed in invertebrates (e.g. annelids) and lower vertebrates such as urodele amphibians [12]. Amphibians constitute an excellent model organism for studies of regenerative pathways since they possess the ability to regenerate and faithfully reconstruct most of their body structures (e.g. the limb, tail, retinal epithelium and lens) [13]. Limb regeneration in urodeles provides the most prominent paradigm of how an entire structure undergoes drastic reversal of phenotype through a series of well-defined stages that include: dedifferentiation, proliferation, and redifferentiation of mesenchymal cells into the specific cell populations that comprise the tissues of the regenerating limb.

The rather surprising role of innate immunity in vertebrate regeneration was first denoted in a study showing that complement component C3 is specifically expressed in the dedifferentiating cell mass and early regenerative zone ('blastema') of the urodele limb [13].

In addition, the presence of C3 in 'blastema' cell cultures expressing typical markers of muscle development indicated that this protein might also participate in the early steps deciding the fate of tissue-committed precursors (e.g. myogenic fibroblasts). This finding implicated complement in early muscle development as such is encountered in settings that evoke extensive muscle reconstruction (e.g. limb regeneration).

Recently the original hypothesis that complement may be an important mediator of regeneration in urodeles has been stirred by findings that also implicate C5 in the processes of lens and limb regeneration in another amphibian species, the newt [14].

COMPLEMENT PROTEINS ARE CRUCIAL TO MAMMALIAN REGENERATION

Unlike the promiscuous regenerative capacity of lower vertebrates, in mammals, the propensity to regenerate has been conserved as an evolutionary vestige restricted to very few organs and tissues. The tissues that tend to self-renew/regenerate are those that are more susceptible to acute environmental perturbations or lie directly exposed to various stress stimuli and toxic agents (e.g. skin, intestinal epithelium). The liver is one of the few organs in the adult body of mammals that has retained the ability to regenerate by replacing its parenchymal cell population in response to various insults, including toxic exposure, viral infection, and surgical resection (e.g. partial hepatectomy) [15]. Several cytokine, hormonal, and growth factor-dependent pathways have been implicated in triggering liver regeneration [16].

Two pro-inflammatory cytokines, IL-6 and TNF- α , are considered among the vital factors that modulate the early stages of hepatocyte regeneration by contributing to the mitogenic priming of hepatocytes and the early growth response of the liver [16]. Downstream signaling cascades activated by these cytokines are thought to be responsible for the recruitment of the hepatic transcription factors NF- κ B and STAT3 that in turn translocate to the nucleus and activate the transcription of a wide array of hepatic growth response genes [17]. This cascade of events results in the synchronized entry of quiescent hepatocytes into the cell cycle and eventually leads to the growth factor-driven proliferation of these 'primed' cells.

However, until recently, the potential interaction of such cytokine-dependent pathways with elements of the innate immune response and the mechanism by which such a molecular 'crosstalk' could affect the priming of hepatocytes and promote liver regeneration had not been rigorously addressed.

The platform for investigating the potential role of innate immune systems in liver regeneration was set by the recent finding that mice lacking C5 were unable to regenerate their liver after acute toxic injury [18]. The absence of C5 as well as the functional ablation of C5aR-

dependent signaling caused severely diminished liver regeneration by abrogating the ability of hepatocytes to reenter the cell cycle. These results indicated that C5 exerts its function on hepatocytes through its activated fragment, the anaphylatoxin C5a, and that the downstream stimulation of C5aR signaling pathways that act either locally, or extrahepatically, might promote the release of 'priming' factors (such as pro-inflammatory cytokines) essential for the growth response of the liver [18].

This hypothesis was strongly endorsed by other independent studies demonstrating that C5a can modulate cytokine release at the hepatocyte-Kupffer cell interface. Indeed, C5aR stimulation appears to modulate the activation of acute phase response genes (e.g. α (2) macroglobulin) in hepatocytes by co-stimulating the release of IL-6 from non-parenchymal liver cells (Kupffer cells) [19]. In addition, it was recently shown that IL-6 can induce the de novo expression of functional C5aR receptors in rat hepatocytes *in vivo* [20]. Therefore, it would be interesting to speculate that an interplay between IL-6 and C5a-dependent pathways might provide costimulatory signals to quiescent hepatocytes and promote their proliferation in settings of acute stress or liver damage.

The definitive role of complement components as essential partners of the early 'priming' network during liver regeneration was established later on in a study monitoring the regenerative response of complement-deficient mice after partial hepatectomy. It was demonstrated that not only C5 but also C3 is required for the regenerative response of these mice. Moreover, both anaphylatoxins, C5a and C3a, were shown to participate in the priming of hepatocytes in a synergistic fashion [5]. Combined administration of recombinant and synthetically derived anaphylatoxins rescued deficient mice from primary hepatic failure and parenchymal injury and effectively restored the BrdU index in S-phase hepatocytes [5].

Furthermore, it became evident that complement mediated pathways are indeed coupled to the cytokine network that drives the early growth response of the regenerating liver. In this respect, blockade of C5aR signaling abrogated regeneration after hepatectomy by disrupting the normal expression profile of cytokines and inhibiting to a great extent the activation of hepatic transcription factors that are essential for the 'priming' of quiescent hepatocytes [5].

Taken together, these findings support a novel role for the complement system as an important modulatory component of hepatic growth and regeneration. In addition, they open a new avenue in therapeutics, suggesting that products of complement activation may prove beneficial reagents amendable to clinical application, in terms of enhancing liver regeneration in clinical liver transplantation settings.

Studies evaluating the stimulatory effect of complement anaphylatoxins on hepatocytes are still in an infant stage, but the *in vivo* findings using animal models of regeneration show great promise and encourage the consideration of such an approach in a clinical setting.

A ROLE FOR COMPLEMENT IN STEM CELL HOMING AND ENGRAFTMENT

A complex interplay between chemokines cytokines, proteolytic enzymes, adhesion molecules, stromal and hematopoietic cells dictates the migration of stem cells to the peripheral blood and to other lymphoid organs, and regulates the homing of these cells to the bone marrow and their anchorage within this organ [21]. Manipulating the homing potential of CD34+ bone marrow-derived precursor cells and devising effective mobilization strategies that enhance the release of hematopoietic stem cells will provide a novel stem cell-based therapeutic platform for enhancing the hematopoietic recovery of bone marrow-transplanted patients and for effectively targeting donor stem cells to specific organs in settings of acute injury or stress.

Although a crucial role for CXCR4-SDF-1 interactions in the homing of stem cells to the bone marrow and their retention within this organ has been recently documented [22], very little is still known about the mechanisms that regulate the homing and engraftment of hematopoietic stem cells.

Various complement proteins, membrane regulators and receptors are expressed by a wide spectrum of hematopoietic cells and this is mainly associated with the protection of these cells from autologous complement-mediated damage and their inflammatory recruitment and activation during the course of infection [23].

Interestingly, a potential role for complement in early hematopoietic development and stem cell homing was suggested in a recent study profiling the expression of various complement components and receptors

in human stem/progenitor cells as well as in lineage-committed hematopoietic precursors. It was shown that human clonogenic CD34⁺ cells express a functional receptor for the C3a anaphylatoxin, and that C3a receptor-mediated signaling co-stimulates the chemotaxis of human CD34⁺ cells by synergistically increasing the migration of these cells in the presence of *a*-chemokine stromal-derived factor-1 (SDF-1) [24]. Furthermore, C3a was found to modulate various homing activities (e.g. adhesion, transmigration) of stem cells by increasing their sensitivity to low doses of SDF-1 [24]. In order to identify the potential source of C3a in the bone marrow, the same study demonstrated the local production of C3 within the bone marrow stroma.

Given that the SDF-1-CXCR4 signaling axis appears to regulate various homing activities of hematopoietic stem cells, an intriguing hypothesis was put forward according to which, C3aR may mediate critical homing functions of hematopoietic stem cells in synergy with the SDF-1-CXCR4 axis. Thereby, C3a and its receptor might regulate processes that affect the SDF-1-dependent migration of stem cells to various organs or modulate the anchorage/retention of stem cells within the bone marrow hematopoietic niches.

This hypothesis was validated by recent studies showing that C3a and its receptor C3aR promote the retention of hematopoietic progenitor/stem cells in the bone marrow of mice that have been subjected to HSC mobilization with daily injections of G-CSF [25]. C3-/- or C3aR-/- mice showed increased release of bone marrow progenitors into circulation following G-CSF-induced mobilization. Furthermore, treatment of normal mice with a C3aR antagonist led to increased release of hematopoietic stem cells (HSC) to the periphery, suggesting that antagonists of the C3aR can serve as a prototype for developing effective mobilizing agents for HSC transplantation in the clinic.

The *in vivo* findings using HSC mobilization protocols in mice have provided compelling evidence for a role of complement in stem cell homing and engraftment. Further studies will have to address the potential application of similar complement-based protocols to transplanted patients.

CONCLUDING REMARKS

From a clinical standpoint, complement research has emphasized on developing therapeutics that can control detrimental effects of complement activation associated with severe inflammatory cascades leading to local or remote tissue damage. Hence, considerable effort has been devoted in the search of effective inhibitors that can control inappropriate activation of this system and prevent the propagation of complement-dependent inflammatory reactions. However, complement has been recently described as a 'double edged' sword that possesses both beneficial and detrimental properties for the host. This article has focused on functions of complement that do not relate to its inflammatory nature and underscore its beneficial role in non-inflammatory and developmental contexts.

Products of complement activation appear to modulate processes that range from early hematopoietic development to tissue regeneration and human reproduction. The anaphylatoxin receptors C3aR and C5aR and their downstream activation partners interact with previously unidentified networks to provide essential signals for cell survival, proliferation and migration in various biological contexts. This surprising versatility of complement proteins urges scientists to revisit the established concept about complement activation and take into consideration a broader spectrum of biologic activities when designing therapeutic strategies directed against a specific 'phenotype' or disease. The abundance of complement transgenic mouse lines and the availability of specific

complement reagents offer a unique opportunity for dissecting the role of complement proteins in such non-inflammatory processes as those described in this article. However, the extent to which the results of animal experimentation will predict the behavior of the system in humans remains to be determined.

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