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## Complement: more than a guard

## How TCR kinetics determine T-cell responses

## C-type lectins on dendritic cells

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# Complement: more than a 'guard' against invading pathogens?

**Dimitrios Mastellos and John D. Lambris**

**Recent studies have indicated that complement proteins might exert novel functions that are distinct from their well-established inflammatory role, by modulating cellular responses and cell–cell interactions that are crucial to early development and cell differentiation. Accumulating evidence suggests that complement might have important roles in diverse biologic processes, ranging from early hematopoiesis to skeletal and vascular development and normal reproduction. Furthermore, it is now becoming evident that complement-regulated pathways interact with other signaling networks and influence the outcome of complex developmental programs, such as limb regeneration in lower vertebrates and organ regeneration in mammals. These findings highlight a previously under-appreciated role of complement and might have important implications in the context of normal development by helping to elucidate the rather obscure role of innate immunity in such cell modulatory pathways.**

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In his quest for a universal interpretation of nature, the Greek philosopher Heracleitus (535–475 BC) maintained that material elements are not always as we perceive them. His quote, 'nature is fond of hiding herself' eloquently epitomizes the concept that biological systems are not insulated and rigid entities but are dynamic and highly interactive networks influencing various physiological responses. This departure from a traditionally reductionistic approach in scientific thinking to a more global appreciation of basic biological processes has led to novel associations between systems and pathways that would otherwise appear unrelated and divergent.

For decades the complement system has been regarded as an effector arm of the innate immune response and has been recognized as a system that performs well-defined functions within a strictly immunological milieu. It has maintained a high degree of phylogenetic conservation among both invertebrate and vertebrate species, having apparently pre-dated the emergence of adaptive immunity [1]. Complement acts as an effector system in host defense against invading pathogens, contributes through its activation products to the release of inflammatory mediators, promotes tissue injury at sites of inflammation, and has been implicated in the pathogenesis of several autoimmune, ischemic and vascular diseases [2]. Moreover, it has recently been established that complement serves as a vital link between innate and acquired immunity by augmenting the humoral response to T-cell-dependent antigens and affecting the threshold of B-cell activation [3].

However, recent studies have provided striking evidence for alternative functions of complement that are rather divergent from its traditional inflammatory role and that until recently, have been under-appreciated. The existence of distinct expression profiles for various complement components in different tissues and developmental stages, and previously uncharted biologic activities that are associated with this expression in various settings support a novel role for this system in cell modulatory pathways that are crucial to early

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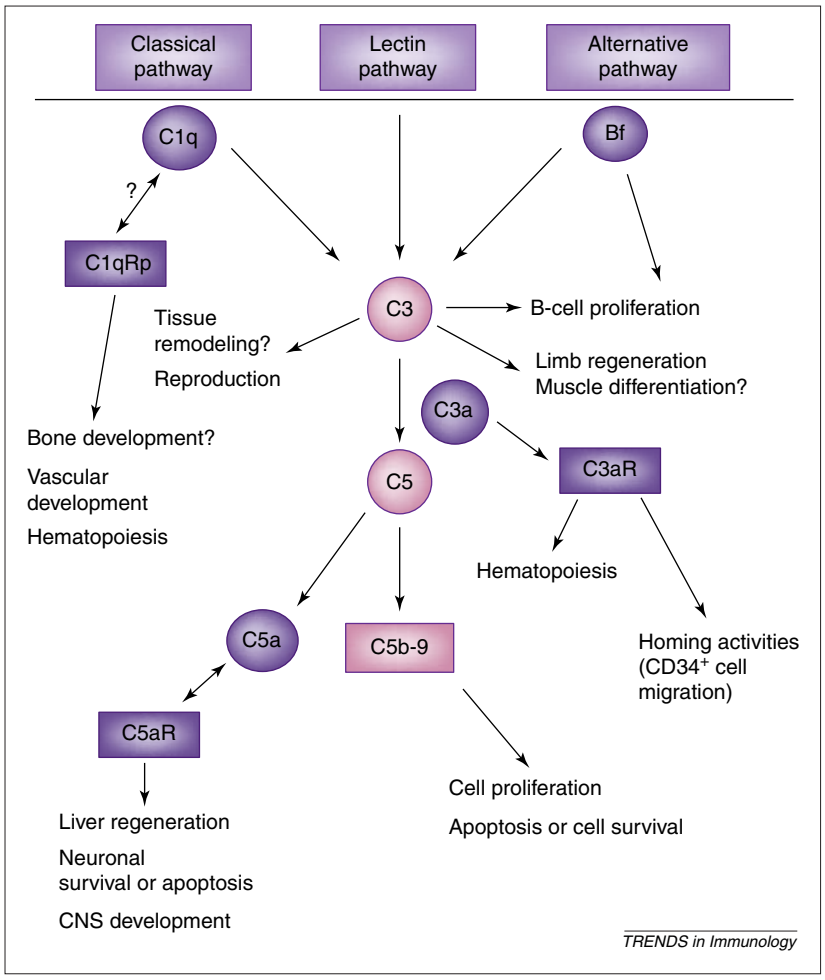


**Fig. 1.** Complement activation triggered by any of the three pathways (alternative, classical or lectin) results in the sequential proteolytic cleavage of proteins and the generation of bioactive fragments and protein complexes that mediate diverse functions in the context of normal development. The various components and complement receptors that have been implicated in the regulation of different developmental processes are shown in this schematic diagram.

development, stem-cell commitment and differentiation, reproduction and tissue and organ regeneration (Fig. 1). Compelling evidence suggests that complement proteins might not only be recruited upon infection and inflammation as an innate defense mechanism, but also might contribute to diverse biologic functions and phenotypes through their functional crosstalk with other cellular networks.

Here we discuss these novel findings and outline plausible mechanistic models that integrate complement-regulated pathways into broader signaling networks underlying developmental processes and distinct cell phenotypic transitions.

## Complement in bone and cartilage development

During bone formation in early fetal development, a sequence of well-defined changes occurs in chondrocytes, leading to a gradual replacement of the cartilaginous matrix by endochondral bone. These changes include proliferation and differentiation of existing chondrocytes, disintegration of hypertrophic chondrocytes in the growth plate, matrix remodeling and differentiation of invading mesenchymal cells into osteoblasts, which ultimately produce the bone matrix that replaces the fetal cartilage [4]. The signals that regulate this transition from cartilage to bone (osteogenesis) and the multiple factors that promote bone and cartilage development remain poorly defined.

Surprisingly, complement has recently been implicated in bone development (Fig. 2) by studies showing that C3 is secreted by bone marrow-derived (ST2) stromal cells and primary osteoblastic cells *in vitro*, after stimulation with vitamin D3. Recent evidence has also suggested that complement components might be involved in pathways that regulate the differentiation of bone marrow-derived progenitors into osteoclasts. In this respect, C3 potentiates the macrophage-colony stimulating factor (M-CSF)-dependent proliferation of macrophage-like mononuclear progenitors and synergistically promotes their differentiation into osteoclasts [5].

In another study the expression of several complement components has been localized to distinct zones of the developing endochondral bone. The distribution pattern of proteins, such as C3, factor B, C5, C9 and properdin in different zones within the developing cartilage has suggested a potential role for complement in cartilage-bone transformation, matrix degradation, bone remodeling and vascularization [6].

In a further development adding to this repertoire of 'non-inflammatory' functions of complement, C1q has recently been implicated as a possible marker for the differentiation of mesenchymal cells into early chondrocytes during skeletal development [7]. By means of suppression subtractive hybridization, a novel gene (*CORS26*) was isolated from a murine cell line stimulated to differentiate along the chondrocytic lineage. Sequence analysis of this gene revealed a striking similarity to human C1q. Its distinct pattern of expression during bone development, together with the finding that COS26 can act as a growth factor for mouse chondrocytic cells *in vitro*, gave rise to the hypothesis that this C1q-like molecule might participate in autocrine or paracrine regulatory pathways that influence chondrocyte differentiation during early skeletal development.

## Complement in mammalian reproduction

The presence of almost all complement components and membrane regulators (CRs) has been documented in epithelial and vascular tissues lining the entire female reproductive tract (including the endometrium, cervical epithelium and Fallopian tubes) [8]. This prominent expression of membrane regulators, such as decay accelerating factor (DAF), membrane cofactor protein (MCP), CD59, CR1 (complement receptor-type 1) on all reproductive epithelia as well as on the surfaces of sperm (e.g. MCP, CD59) and oocytes (e.g. MCP, CR1) has thus far been associated with the protection of these tissues from autologous complement activation and injury. Recently, the beneficial role of CRs in warding

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off aberrant complement activation at the placental interface was underscored by a study showing that *crry*, which encodes a murine complement regulator, is crucial to maintaining feto-maternal tolerance during early pregnancy [9].

Interestingly, there is increasing evidence that the biosynthesis of several complement components and receptors in the reproductive tract is under fine hormonal regulation and that it follows stage-specific expression patterns during the menstrual cycle and might thus contribute to normal reproductive processes [10]. For instance, C3 synthesis by the glandular epithelium of the human and rat uterus is estrogen-driven. Furthermore, factor B and DAF are synthesized in the human and rat endometrium in a hormone-dependent manner, and distinct changes in the expression of various complement membrane regulators occur during gamete maturation, follicular development and ovulation [10].

A fascinating aspect that has remained rather elusive in the field of complement research is the potential involvement of complement in early stages of the sperm–oocyte interaction, leading to gamete fusion and fertilization. Mammalian fertilization consists of a series of events, including the sperm acrosomal reaction that leads to the release of proteases into the sperm–oocyte space, the penetration and attachment of sperm to the oocyte plasma membrane by receptors expressed on the sperm surface, and finally the sperm–oocyte fusion [11].

Human sperm that have undergone the acrosomal reaction express nascent MCP receptors on the sperm

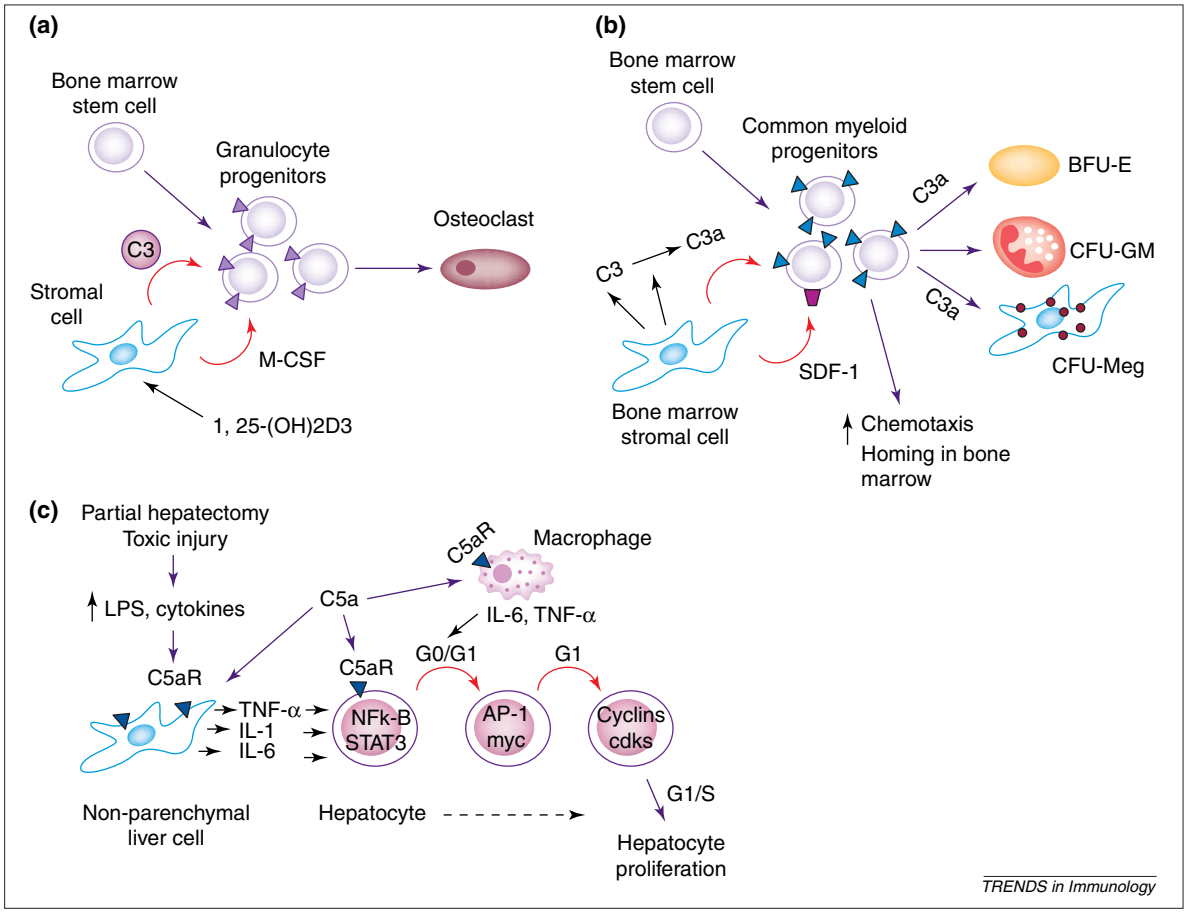


**Fig. 2.** Schematic illustration of three hypothetical models outlining the potential role of complement proteins in different developmental programs. (a) The role of complement component C3 in osteoclast differentiation during bone development. Bone marrow-derived stromal cells secrete C3 in response to vitamin D3. Binding of C3 to receptors expressed on granulocyte progenitor cells, in the presence of macrophage-colony stimulating factor (M-CSF), synergistically promotes the differentiation of these precursors into osteoclasts. The purple triangles represent a putative C3-binding protein. (b) Potential effects of complement component C3 and the C3a anaphylatoxin receptor, C3aR, in hematopoietic development. The C3a receptor is expressed by human $CD34^+$ stem cells and bone marrow-derived stromal cells have been shown to secrete C3. Local generation of C3a appears to drive the maturation and lineage commitment of various hematopoietic progenitors. A synergistic interaction between CXCR4 and C3aR (and their respective ligands, SDF-1 and C3a) might influence the homing of hematopoietic progenitors to the bone marrow. The pink trapezoid represents the CXCR4 chemokine receptor and the blue triangles represent the C3a anaphylatoxin receptor. (c) Contribution of complement to liver regeneration: The anaphylatoxin C5a might promote cell-cycle re-entry and proliferation of hepatocytes through the recruitment or activation of cells that bear C5a receptors (C5aR): i. by binding to the C5aR in non-parenchymal liver cells and modulating the release of cytokines that prime hepatocytes to proliferate; ii. by directly stimulating the growth of hepatocytes by the inducible, interleukin (IL)-6 dependent, expression of C5aR on their surface and iii. by mediating the release of cytokines or other 'priming' factors from blood-derived macrophages which subsequently stimulate hepatocyte proliferation. Abbreviations: AP-1, activator protein-1; BFU-E, erythroid progenitors; cdks, cyclin-dependent kinases; 1′α, 25-$(OH)_2D_3$, 1′α, 2,5-dihydroxyvitamin $D_3$; CFU-GM, granulocyte progenitors; CFU-Meg, megakaryocytic progenitors; CXCR4, chemokine receptor; SDF-1, α-chemokine stromal-derived factor-1; STAT3, signal transducer and activator of transcription 3.

acrosomal membrane [12]. In addition to acting as a cofactor for factor I-mediated cleavage of C3 and thereby ensuring complement inactivation and protection of sperm from injury, sperm MCP appears to possess distinct properties associated with the sperm–oocyte interaction. In support, monoclonal antibodies directed against MCP can inhibit the penetration of hamster oocytes by human sperm [12]. Furthermore, C3 might also be involved in the early steps of gamete interaction and fusion. Evidence showing that sperm acrosomal enzymes released during the acrosomal reaction are able to cleave C3 has given rise to the hypothesis that C3-derived fragments such as C3b and iC3b might serve as bridging ligands between MCP receptors expressed on the sperm and C3 receptors (CR1, CR3) present on the plasma membrane of the oocyte [12]. In this way C3 and C3-binding proteins would facilitate gamete membrane fusion and thereby promote sperm penetration and oocyte fertilization. Evidence suggesting a similar role for C3 and complement receptors in amphibian fertilization has provided additional credence to this hypothesis and further implies that a link between complement and reproduction might have been established early in evolution [13].

All of these studies have implicated complement in normal reproduction and raised the intriguing possibility that innate immune pathways might not only confer protection on host tissues from autologous inflammatory damage but also might influence specific hormone-regulated pathways that underlie gamete development, maturation and fertilization.

## Complement in tissue and organ regeneration

### *A role for C3 in urodele regeneration*

The ability to regenerate complex structures and reconstruct entire body parts from damaged tissues is a trait widely encountered among invertebrates (e.g. annelids, hydroids) and in lower vertebrates, such as amphibians [14]. In urodele amphibians (axolots, newts) the process of regeneration is particularly prominent in the limb, tail and in structures of the eye (retinal epithelium and lens) [14]. Limb regeneration in urodeles entails the activation of complex developmental pathways that act in concert to promote the dedifferentiation, proliferation and redifferentiation of mesenchymal cells into the specific cell types that comprise the various tissues of the regenerating limb.
A morphogenetic hallmark in the process of limb regeneration is the formation of the blastema, a cell layer consisting of dedifferentiated epithelial and mesodermal cells that gradually receive signals from the local microenvironment (e.g. wound epithelium), re-enter the cell cycle and undergo differentiation into the various cell types that will reconstitute the muscle, bone and connective tissue of the regenerating limb [15].

A surprising link between complement biosynthesis and tissue regeneration was first suggested in a recent study showing that C3 is specifically expressed in the blastema cell layer of the regenerating limb in urodeles [16]. Interestingly, C3 was also detected in blastema cell cultures of the myogenic lineage, a finding that suggests that C3 might be crucial to muscle development and myoblast differentiation in settings that evoke extensive muscle reconstruction, such as limb regeneration. Moreover, the fact that C3 could not be detected in similar structures within the normally developing limb of urodeles further corroborates the hypothesis that this complement component plays a specific role associated with the regenerative process [16]. A recent finding that further supports this concept is the delayed limb regeneration that is observed in newts after treatment with the complement-depleting agent, cobra venom factor (CVF) (Y. Kimura *et al*., unpublished).

The distinct expression pattern of C3 in tissues that undergo extensive remodeling during limb regeneration (e.g. muscle) has led to the speculation that complement proteins might serve as important mediators of tissue regeneration in part by promoting cell–cell interactions that are crucial to matrix degradation and tissue remodeling. C3 and its activation fragments are capable of interacting with several components of the extracellular matrix (such as laminin, fibronectin and integrins) [17], with integrin-like complement receptors (e.g. CD11b or CD18) that mediate cell adhesion and cell–cell communication [18], and with G-protein coupled receptors that induce cell motility and migration (e.g. the C3a anaphylatoxin receptor C3aR) [19]. These functions are essential to normal tissue regeneration, a process that culminates in the coordinated spatiotemporal activation and redistribution of various cell populations within the regenerating zone. These mechanisms by which C3 might contribute to limb regeneration, although plausible, remain speculative and further research is required to elucidate the precise role of complement in this complex developmental process.

## C5aR-mediated pathways implicated in mammalian liver regeneration

The observation that C3 is expressed in regenerating tissues of the urodele limb gave rise to the fascinating concept that complement components might have been selected through evolution as important mediators of tissue regeneration, not only in lower vertebrates but also in more evolved species.

The liver is one of the few quiescent organs in the adult body of mammals that has retained the ability to regenerate and restore its homeostasis in response to various perturbations, including toxic exposure, viral infection and surgical resection (e.g. partial hepatectomy) [20]. Several cytokine, hormonal and growth factor-dependent pathways have been

implicated in triggering liver regeneration [21]. It is currently accepted that interleukin (IL)-6 and tumour necrosis factor (TNF)-α are among the crucial factors that regulate the early stages of liver regeneration by contributing to the mitogenic priming of hepatocytes and the early growth response of the liver [21]. Although liver regeneration has been well documented as a physiological response to various stimulants, the wide array of molecular mechanisms and early signals that underlie its transition from quiescent to regenerative phenotype still remain undetermined.

The liver constitutes the primary site of biosynthesis for most of the complement components [2]. The role of complement in modulating hepatic immune surveillance and maintaining innate host defense against ingested pathogens that are transferred through the portal circulation to the hepatic microenvironment is well appreciated [22]. Interestingly, recent evidence has attributed novel functions to complement that are not clearly placed within an inflammatory context in the liver, by suggesting that complement components might be essential for liver regeneration in settings of acute toxic injury. In a recent study involving C5-deficient mice, liver regeneration was shown to be severely impaired after acute toxic injury, and hepatocyte proliferation was abrogated in the absence of C5 or when C5a receptor stimulation had been ablated [23]. These results indicated that C5 exerts its function on hepatocytes through its activated fragment C5a, and the downstream stimulation of C5aR signaling pathways that act either locally, in a paracrine manner, or extrahepatically, promoting the release of 'priming' factors (such as proinflammatory cytokines) from circulating leukocytes [23].

Other independent studies have corroborated the involvement of complement in liver regeneration by demonstrating that C5a can modulate cytokine release at the hepatocyte–Kupffer cell interface. In support of this concept C5a can act synergistically with lipopolysaccharide (LPS) and activate genes involved in the acute phase response (e.g. α(2) macroglobulin) in hepatocytes by costimulating the release of IL-6 from non-parenchymal liver cells (Kupffer cells) [24]. LPS is considered one of the earliest priming factors involved in the initiation of liver regeneration [20], however, the precise mechanism by which LPS participates in the regenerative response of the liver remains poorly understood. The recent finding that C5a can potentiate the LPS-dependent release of IL-6 in the liver provides a potential link between LPS and C5aR-mediated pathways leading to cytokine modulation. It is therefore reasonable to speculate that a similar pathway involving C5a-mediated stimulation of IL-6 could also be recruited during liver regeneration and provide hepatocytes with the essential signals to re-enter the cell cycle.

In another interesting development, IL-6 can induce the expression of functional anaphylatoxin C5aR receptors in rat hepatocytes *in vivo* [25]. This finding provides further mechanistic links between complement C5aR stimulation and cytokine regulation in the hepatic microenvironment, thus underlining the complexity and dynamic nature of these interactions (a hypothetical model of these interactions is outlined in Fig. 2).

From a different standpoint, C5a has recently been shown to indirectly enhance glucose output from rat hepatocytes by stimulating the release of prostaglandins from Kupffer cells [26]. The bioavailability of glucose in the early stages of liver regeneration ensures that hepatocytes can overcome the metabolic threshold required for cell cycle re-entry and proliferation. Therefore, a possible role for C5a in maintaining the necessary glucose balance and metabolic stability of hepatocytes during liver regeneration cannot be ruled out.

Taken together, these findings support a novel role for C5 in liver regeneration and strongly implicate the complement system as an important immunoregulatory component of hepatic growth and homeostasis. In addition, they highlight the concept that complex processes, such as liver regeneration, are multidimensional, requiring the synergistic interaction of diverse pathways and the integration of signals that originate both locally and extrahepatically. The recruitment of such systems that act in parallel essentially provides the liver with functional redundancy and ensures its complete recovery and metabolic integrity under various settings of injury, infection or severe mass deficit (such as liver transplantation).

### A role for complement in hematopoiesis and vascular development

The expression of various complement proteins, membrane regulatory molecules and receptors by a wide spectrum of blood-cell types has been well documented and is mainly associated with the protection of these cells from complement-mediated lysis and their inflammatory recruitment and activation during the course of infection [27]. Very little is known, however, about the distribution of complement components in early hematopoietic progenitor cells, their potential role in the commitment of different precursors to various lineages during hematopoietic development, and the complement-mediated interactions that influence the homing of lymphoid progenitors in various tissues.

Recent studies suggest an intriguing role for the phagocytic complement C1q receptor (C1qRp/CD93) in early hematopoietic development [28]. In this respect, a novel fetal stem-cell antigen (AA4) was recently identified as the mouse homologue of human C1qRp. During early embryogenesis the AA4 antigen is predominantly expressed in vascular endothelial cells, vessel-associated hematopoietic clusters and hematopoietic progenitors located in the fetal liver. Its expression pattern remains localized within the

**Table 1. Complement components modulate cell survival and/or proliferation**

| Complement component | Target cell | Species | Receptor | Cellular response | Refs |
|---|---|---|---|---|---|
| C3 | B lymphocytes | Human | CR2 | Proliferation | [30] |
| Factor B (Bb) | B lymphocytes | Human | ? | Proliferation | [31] |
| C5a | Thymocytes | Rat | C5aR | Apoptosis | [32] |
| C5a | Neuroblastoma cells | Human | nC5aR | Apoptosis | [33] |
| C5a | Neuroblastoma cells | Human | nC5aR | Cell survival, proliferation and differentiation | [34] |
| MAC | Oligodendrocytes | Rat | – | Survival, dedifferentiation and cell cycle entry – reduction in expression of myelin-associated proteins (e.g. MBP, PLP) | [35] |
| MAC | Skeletal muscle cells | Rat | – | Dedifferentiation and cell cycle entry – reduction in expression of muscle- specific proteins (e.g. a-actin, troponin) | [35] |
| MAC | Aortic smooth muscle cells | Human | – | Cell cycle entry–proliferation | [35] |
| MAC | Schwann cells | Rat | – | Proliferation and rescue from apoptosis | [35] |
| MAC | Fibroblasts (3T3) | Mouse | – | Proliferation | [36] |

Abbreviations: nC5aR, neuronal C5aR; MAC, Membrane attack complex (C5b-9); MBP, myelin basic protein; PLP, proteolipid protein.

hematopoietic compartment (i.e. in subsets of $CD34^+$ stem cells in the bone marrow) of the adult mouse, but it also expands to other organs and is particularly prominent in the vascular endothelium of the lung and heart [28].

This study suggests for the first time that the AA4 stem-cell marker, and potentially also its human homologue C1qRp/CD93, might participate as adhesion molecules in crucial cell–cell interactions taking place during early hematopoietic development, such as the homing of lymphoid progenitors in various tissues. Furthermore, the abundant expression of AA4 in endothelial cells in both early fetal development and adult mouse tissues (heart) indicates a possible role for AA4 in angiogenesis and blood vessel remodeling by mediating cell–cell interactions during endothelial cell migration, invasion and proliferation into the vascular space [28].

The potential involvement of C1q in such developmental processes, as a ligand for C1qRp/CD93, remains to be clarified because the nature of this interaction is currently controversial [29]. However, the possibility that C1qRp mediates its effects during early hematopoiesis and vascular development through its interaction with an alternative, yet unidentified ligand, cannot be excluded.

Additional support for a novel role of complement in hematopoietic development and stem-cell differentiation comes from a recent study profiling the expression of various complement components and receptors in normal human early stem or progenitor cells as well as in lineage-committed hematopoietic cells. In particular, the G-protein-coupled receptors for both C3a and C5a anaphylatoxins were found to be expressed by human clonogeneic $CD34^+$ cells, and complement components C3 and C5 were both found to be secreted by the bone marrow stroma (R. Reca *et al.*, unpublished).

Furthermore, there is evidence suggesting that the C3a anaphylatoxin has a role in the maturation and lineage commitment of hematopoietic progenitors (Fig. 2), because in serum-free cultures it was found to costimulate the development of cells within the erythroid and megakaryocytic, but not granulo-monocytic, lineages (R. Reca *et al.*, unpublished).

In addition, stimulation of the C3aR appears to regulate the chemotaxis of human $CD34^+$ cells by synergistically increasing the migration of these cells in the presence of α-chemokine stromal-derived factor-1 (SDF-1) (R. Reca *et al.*, unpublished).

The striking observations that C3, like SDF-1, is secreted by bone marrow-derived stromal cells, and that both C3aR and CXCR4 are expressed by human $CD34^+$ cells have laid the groundwork for further investigation of the hypothesis that a functional cross-talk between the C3aR and CXCR4 signaling pathways might have an important role in the homing of human stem or progenitor cells to the bone marrow hematopoietic niches.

## Concluding remarks

Apart from their well established role in the inflammatory response, several complement components and their activation products have recently been suggested to mediate novel, non-inflammatory functions in various tissues (Table 1). C3 has been shown to promote growth of CR2-positive lymphoblastoid B cells *in vitro* [30]. A similar effect on B-cell survival has been reported for other components, such as factor B [31]. In addition, several studies have implicated the anaphylatoxic fragment C5a in pathways that regulate cell apoptosis or proliferation [32,33]. C5a has been shown to be mitogenic for undifferentiated human neuroblastoma cells and also appears to protect terminally differentiated human

neuroblastoma cells from toxicity mediated by the amyloid Aβ peptide [34]. Taken together, these studies suggest an intriguing role for neuronal C5aR receptors in CNS development.

Assembly of the membrane attack complex (MAC) on the cell surface of complement-targeted cells has traditionally been associated with osmotic lysis and cell elimination. Recently, however, it has become evident that exposure of cells to sublytic doses of the MAC can trigger diverse intracellular responses affecting pathways of cell survival, proliferation or apoptosis [35,36] (Table 1). Because similar intracellular substrates are activated upon growth-factor receptor stimulation in various cells, it would be interesting to speculate that complement- and growth factor-regulated pathways might functionally overlap at the cell membrane interface and modulate intracellular responses through the activation of convergent downstream effectors.

Complement has long been perceived as merely an effector arm of the innate immune response and as an inflammatory mediator in various diseases. Here we present recent findings that implicate complement in novel, non-inflammatory processes and developmental pathways. These findings provide a framework for the further study of complement-mediated effects on cell survival and proliferation. They also have important implications for normal developmental processes that entail activation, cell cycle re-entry and proliferation of quiescent, terminally differentiated cells, as illustrated in the case of organ regeneration or tissue remodeling. The advent of transgenic technology has enabled the generation of several complement-deficient mouse strains over recent years and has contributed to elucidating basic biologic responses mediated by complement. However, no rigorous investigation has been performed to evaluate the ability of these animals to support developmental processes such as hematopoiesis, bone, vascular and cartilage development. Furthermore, one could anticipate that these complement deficiencies would not cause noticeable defects under steady-state conditions because of a likely compensatory effect exerted by other pathways in these mice. In conclusion, it would be interesting to speculate that complement provides a vital 'innate immune' partner in development that is recruited to promote cell differentiation, tissue remodeling and regeneration under stress-related conditions and in response to acute environmental perturbations.

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